2nd European Symposium on Porcine Health Management

- Pig Health, Performance and Welfare -

2nd ESPHM

Proceedings

26th - 28th May 2010
Hannover, Germany

ECPHM EUROPEAN COLLEGE OF PORCINE HEALTH MANAGEMENT

Proceedings

The 2nd European Symposium on Porcine Health Management

27 – 28 May 2010

Hannover, Germany

Edited by
Elisabeth grosse Beilage, Thomas Blaha
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Preface

On behalf of the European College of Porcine Health Management (ECPHM), it is our pleasure to welcome you to the 2nd European Symposium on Porcine Health Management (ESPHM) in Germany at the University of Veterinary Medicine Hannover, Foundation.
The 1st ESPHM took place in 2009 in Denmark at the Faculty of Life Sciences in Copenhagen. Jens Peter Nielsen, President of the 1st ESPHM, wrote in his PREFACE to the first ESPHM Proceedings that the Danish organisers "…hope the 1st ESPHM will be the first of a long line of similar meetings, and hereby create an important way of exchanging knowledge and stimulating cooperation within the porcine health management at the international level". And indeed, the 1st ESPHM in Copenhagen was not only a very successful scientific meeting, but the first step of the long journey to develop a well established series of meetings that is to become a MUST for every veterinarian specialised in pig diseases and porcine health management. There is a saying that "even the longest journey starts with the first step", to which we want to add "yes, but the second step needs also to be done, otherwise…" Therefore we built on Copenhagen and organised the 2nd ESPHM to see, whether the veterinary pig specialists throughout Europe and maybe beyond Europe regard annual meetings on porcine health management something that is welcome and needed.

We think the fact that we received more than 100 posters submitted (which is more than double the number of the posters for the 1st ESPHM) and more than 230 registrations is a clear message from the professional world dealing with pig health, pig welfare and pork safety that a European equivalent to the annual meetings of the American Association of Swine Veterinarians (AASV) is filling a gap.

The scientific sessions of the 2nd ESPHM "Pig Welfare", "Production and Performance", "Herd Health Programmes", and "Diagnostics in Porcine Health Management" reflect that dealing with the single diseases is not any longer the focus of "pig veterinarians", but the focus is shifting more and more to prevention, improving health and proactive diagnostics. This follows the increasing demand of the society and the ever changing legislation to improve the health and welfare of our food producing animals and of reducing the disease level with simultaneously reducing the liability of animal production on the routine use of antimicrobial substances.
Apart from exchanging cutting edge knowledge on porcine health management, the European College of Porcine Health Management with its currently more than 120 ECPHM Diplomates, its 10 permanent residency programmes and more than 10 residents being trained and examined for becoming ECPHM Diplomates is especially dedicated to promoting our young colleagues that strive to become specialists in the brought field of pig health and pork safety. Therefore, the 2nd ESPHM has also a session, during which our current ECPHM residents are to present their scientific programmes and to discuss with the audience their acquired knowledge. The same is true for the newly introduced poster discussion session, during which the authors of scientifically outstanding posters are asked to present their work and their results.

There is another aspect that makes the 2nd ESPHM not only the "second step of the long journey", but also an increase of the degree to which the pig veterinarians of Europe are organised: in the evening of the first day of the 2nd ESPHM, the founding of the European Association of Porcine Health Management (EAPHM) will take place. This European organisation for pig veterinarians of all disciplines, especially the practitioners, is to represent the interests and to serve the needs of all veterinarians specialised in pig and pork production throughout Europe. Its vision is:

- Promoting a competitive and sustainable European pig production by creating and further developing common guidelines for porcine health management in Europe in order to optimise professional standards leading to the highest possible animal health status for improving food safety, public health and animal welfare in pig and pork production,
- Improving and standardising the undergraduate and post-graduate education, and the life-long learning in the area of porcine health management in Europe, and
- Becoming the opinion leader throughout Europe in the field of porcine health management.

Thus, we hope that the 2nd ESPHM with all its activities, generously sponsored by the industry (see the logos of our sponsors at the back of these proceedings), will be a success in terms of a scientific and social win-win event for all participants and for ESPHM as well for the new EAPHM.

Thomas Blaha
President 2nd ESPHM

Elisabeth grosse Beilage
Chair of the Scientific Committee

Acknowledgements

The 2nd ESPHM 2010 in Hannover would not have been possible without the financial and professional support of our Symposium Partners and Sponsors.

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We also would like to thank those who have submitted papers for providing the latest scientific and practical information for researchers, practitioners and other members of the pig community. A special thank goes to those who have prepared the keynote lectures and invited lectures on selected important topics. Exchange of new information is the core business of the symposium.

The Scientific and Organising Committee, the European College of Porcine Health Management (ECPHM) and the University of Veterinary Medicine Hannover is thanked for assistance and support throughout the planning and organising process.

Scientific Programme

Thursday, May 27, 2010

08:00 - 09:30	**Annual General Meeting (AGM) of the European College for Porcine Health management (ECPHM)**	
09:30 - 10:00	**Coffee break**	
10:00 - 10:30	**Opening Ceremony**	
10:00 - 10:15	Thomas Blaha	President of the 2nd ESPHM
10:15 - 10:30	Gerhard Greif	President of the TiHo-Hannover
10:30 - 12:30	**Session I: Pig Welfare** Chair: Thomas Blaha	
10:30 - 11:15	Bryan Jones	**Keynote lecture:** Welfare quality
11:15 - 11:30	Karl Heinz Waldmann	Piglet castration
11:30 - 11:45	Xavier Manteca	Welfare, behavior, pain
11:45 - 12:00	Albert Sundrum	Organic pig husbandry systems
12:00 - 12:30	PANEL DISCUSSION	
12:30 - 14:00	**Lunch**	
14:00 - 16:00	**Session II: Pig Reproduction & Performance** Chair: Dominiek Maes	
14:00 - 14:45	Olli Peltoniemi	**Keynote lecture:** Managing the high producing sow during lactation and estrus
14:45 - 15:00	Johannes Kauffold	Utilizing ultrasonography for troubleshooting in reproduction
15:00 - 15:15	Nicoline Soede	Second litter syndrome
15:15 - 15:30	Dagmar Waberski	Optimizing insemination
15:30 - 16:00	PANEL DISCUSSION	
16:00 - 16:30	**Coffee break**	
16:30 - 17:15	**Session III: Posters** Chair: Arlette Laval	
16:30 - 16:40	Jesus Osorio	Brachyspira hyodysenteriae clones dissemined within different European countries
16:40 - 16:50	Arda Aydin	Sound analysis for health monitoring in commercial piggeries
16:50 - 17:00	Nicolas Rose	Modelling the influence of husbandry and control measures on PCV2 dynamics within a farrow-to-finish pig farm
17:00 - 17:10	Guillaume Perreul	Porcine Circovirus Type 2 (PCV2) prevalence in abortions in France
17:15 - 18:30	**Plenary meeting "European Association for Porcine Health Management" (EAPHM)**	

Scientific Programme

Friday, May 28, 2010

08:30 - 10:15	**Session IV: ECPHM residents** Chair: Elisabeth grosse Beilage	
08:30 - 09:15	Katharina Stärk	**Keynote lecture:** Reporting clinical trials
09:15 - 09:30	Verena Gotter	Bacteriological findings in pig herds with high or low Salmonella antibody prevalences
09:30 - 09:45	Alfonso Lopez	Effect of organic selenium in the diet on sperm quality of boars
09:45 - 10:00	Frederic Vangroenweghe	Recent antimicrobial sensitivity data of *Brachyspira hyodysenteriae* in Belgium: An analysis of evolution
10:00 - 10:15	Ken Pedersen	Quantitative assessment of *Lawsonia intracellularis* by PCR in outbreaks of acute diarrhea in weaners
10:15 - 10:45	**Coffee break**	
10:45 - 12:45	**Session V: Pig Herd Health Programs** Chair: Mari Heinonen	
10:45 - 11:30	Jens Peter Nielsen	**Keynote lecture:** Quality assurance (QA) in veterinary practice
11:30 - 11:45	Heiko Nathues	A valid diagnosis requires *Good Clinical Practice* and *Good Laboratory Practice*
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12:00 - 12:15	Carlos Pineiro	QA in Spanish pig production
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14:15 - 16:15	**Session VI: Diagnostics in Porcine Health Management** Chair: Paolo Martelli	
14:15 - 15:00	Joaquim Segales	**Keynote lecture:** New approaches to diagnosis
15:00 - 15:15	Thomas Blaha	Monitoring via meat juice serology
15:15 - 15:30	Luc Mieli	Test validation
15:30 - 15:45	Mathias Greiner	Validation without gold standard
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	Thomas Blaha	President of the 2nd ESPHM
	Olli Peltoniemi	President of the 3rd ESPHM

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MANAGING PIG HERDS

WELFARE QUALITY

Harry J. Blokhuis and R. Bryan Jones

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Introduction

The Welfare Quality® project started in 2004 and became the largest piece of integrated research work yet carried out in animal welfare in Europe. The project is a partnership of 40 institutions in Europe and, since 2006, four in Latin America. The partners are based in 13 European countries and four Latin American (Uruguay, Brazil, Chile and Mexico).
During the project's lifetime our original ideas (c.f. Blokhuis et al., 2003) evolved and the priorities were slightly modified accordingly. However, the general aims have remained the same:

- to develop practical strategies/measures to improve animal welfare,
- to develop a European standard for the assessment of animal welfare ,
- to develop a European animal welfare information standard,
- to integrate and interrelate the most appropriate specialist expertise in the multidisciplinary field of animal welfare in Europe.

Although countries outside Europe are involved, obviously this EU funded project mainly focuses on the European situation.
During the last decades of the 20th century major changes took place in animal production (cf Blokhuis et al., 1998). Production intensified enormously and farms became highly specialised (Porcher, 2001). This development led to a huge increase in the number of animals per farm and to striking increases in actual production. Furthermore, housing conditions and management practices changed profoundly with increased mechanisation and other technological developments. Animal production became increasingly industrialised, with quantity often taking precedence over quality.
Over the years, cultural, attitudinal and commercial barriers hampered constructive communication between farmers and the people who ultimately eat what is produced. The activities of consumer groups and animal protectionists and, more recently, the effects of crises such as swine fever, BSE, foot-and-mouth disease and avian influenza have led to people becoming increasingly aware that animal production is more than just an industry. Issues such as animal welfare, food quality, food safety and the environment have assumed much greater importance for the public ('consumer concerns').
Farm animal welfare is now clearly an important issue for ordinary people across Europe and there is clear demand for higher animal welfare standards (see Eurobarometer, 2005; 2007; Kjaernes and Lavik, 2008).
From the start, Welfare Quality® took on board the results from a sociological study carried out in Europe that included an analysis of consumers' reluctance to purchase animal friendly products (Miele and Parisi 2000; Harper and Henson 2000). This study revealed that an important reason is the lack of transparent, reliable and easily understandable information about the way in which animal-based food products are actually produced. Furthermore, worldwide marketing strategies "confirm that producers and retailers today are ready to apply new criteria so as to provide consumers with extra value" (European Commission 2002).

Welfare Quality® therefore set out to deliver reliable, science-based, on-farm welfare assessment systems for poultry, pigs and cattle as well as a standardised system to convey welfare measures into easy to understand product information.
It was also recognised that a large European effort in the area of animal welfare should also include research designed to identify practical ways of solving some of the main welfare problems in current animal production. Therefore, Welfare Quality® initiated appropriate studies in important areas like handling stress, injurious behaviours, lameness, temperament etc.
In our view an integrated European approach provides a firm basis for the harmonisation of assessment and information systems. It is also considered extremely relevant for the provision of transparent consumer information and for marketing and trade.

Adressing consumer concerns

In Welfare Quality® we aim to address welfare concerns and to allow clear communication about the animals' quality of life and profiling of products. The latter is obviously essential in order to connect animal husbandry practices to informed animal product presentation and purchasing. In a truly integrated effort Welfare Quality® combined analyses of consumer/citizen perceptions and attitudes with existing knowledge from animal welfare science and thereby identified 12 areas of concern that should be adequately covered in the measurement systems (Keeling and Veissier, 2005). These are presented in table 1 as welfare criteria, where the direction for maximising welfare is indicated. Each criterion covers a separate aspect of good animal welfare and the list was chosen to encompass all potential areas of concern while at the same time keeping the total number of criteria to a minimum. To further reduce the number of items and ease the understanding, we group them into 4 classes, called principles in the table, corresponding to the questions:

- Are the animals properly fed and supplied with water?
- Are the animals properly housed?
- Are the animals healthy?
- Does the behaviour of the animals reflect optimised emotional states?

Table giving welfare principles and criteria (from Keeling and Veissier, 2005)

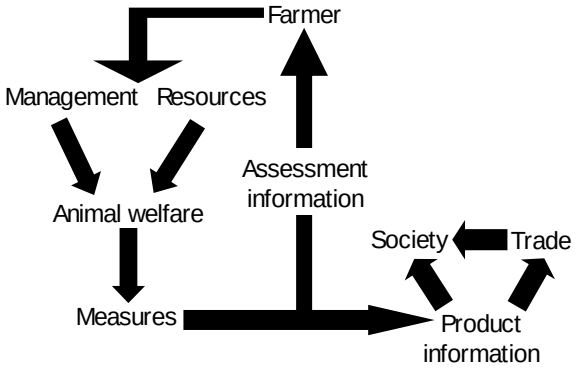
Principles	**Welfare criteria**	
Good feeding	1.	Absence of prolonged hunger
	2.	Absence of prolonged thirst
Good housing	3.	Comfort around resting
	4.	Thermal comfort
	5.	Ease of Movement
Good health	6.	Absence of injuries
	7.	Absence of disease
	8	Absence of pain induced by management procedures
Appropriate behaviour	9.	Expression of social behaviours
	10.	Expression of other behaviours
	11.	Good human-animal relationship
	12.	Positive emotional state

To investigate how animal welfare concerns are relevant for citizens whilst shopping for food and what kind of information is considered relevant for assessing the 'animal friendliness' of the products available on the market focus groups interviews with consumers were carried out in seven study countries (Italy, France, Hungary, UK, the Netherlands, Norway and Sweden) (Miele and Evans, 2005). The results showed that the participants in the focus group discussions reacted favourably to the 'experts' list of areas of concerns in the table above. Most participants identified more commonalities than differences between their understanding and the scientific approach to what is important in defining the welfare of animals.

Welfare Assessment

Most of the currently used welfare assessment systems are largely based on observations of the environment, i.e., design measures presumed to affect animal welfare. However, the links between specific design measures and the animals' welfare status are not always clearly understood. Therefore, one of the main thrusts of the Welfare Quality® project is to develop sets of measures that are animal based i.e. measuring at the animal itself. Such animal-based measures reflect the effects of variations in the way the farming system is managed (role of the farmer) as well as specific system-animal interactions (see diagram below). The measures address all of the above mentioned concerns.

Design measures are also included so that causes of poor welfare can be identified and remedial measures proposed (feed-back to farmer). For each of the different species measures were analysed for validity, repeatability and feasibility and selected for inclusion in the assessment system.



Diagrammatic representation of the measuring and information systems (adapted from Blokhuis et al. 2003).

Welfare Quality® assessment systems for laying hens, broilers, dairy cows, beef cattle, calves, fattening pigs and sows have now been

tested and described in protocols (Welfare Quality®, 2009a, b, c).

Welfare Improvement strategies

In the conception phase of Welfare Quality® it was recognised that a large European effort in the area of animal welfare should also include research designed to identify practical ways of solving some of the main welfare problems in current animal production. Therefore, we initiated appropriate studies in important areas like handling stress, injurious behaviours, lameness, temperament etc. and some very relevant and interesting results are already emerging. The practical improvement strategies that these studies are generating will provide valuable support to farmers and the animal industry in their efforts to improve animal welfare. Since these studies are an integrated part of the Welfare Quality® approach they will also inform and be guided by the assessment information emanating from the welfare assessment system (see diagram above).

Concluding remarks

Even if Welfare Quality® was the largest ever collaborative project in animal welfare science, it is clear it could not have covered all the questions and every detail. So, it is not surprising that there are still unanswered questions and discussion points about specific welfare measures or the lack of animal based measures for some criteria (e.g. no animal based measure for prolonged thirst on the farm nor for thermal comfort in adult cattle). We also had to prioritise some tasks and species at the expense of others because of budgetary and other constraints. Thus we were unable to fully develop the models for integrated assessment for all animal types (e.g. not for sows and piglets on farm, laying hens on farm, and not at all for animals at slaughter). However, the necessary processes and principles have been developed so it is now just a matter of securing the support to carry out the work.

Another gap relates to species and types of animals that could not be included in Welfare Quality®. The other animals that clearly merit study include a number of domestic species such as sheep, horses and turkeys. These are all very important for the continued agricultural and rural development in Europe.

Fittingly, within the context of the Seventh Framework Programme (FP7) the EU recently called for research proposals to further develop and refine the welfare assessment and monitoring system and bring other important species into the model (European Commission, 2009).

During its lifetime the Welfare Quality® project has generated a multitude of results including an innovative way of assessing animal welfare in an integrative way, several concrete strategies to improve animal welfare, many insights into the concerns, initiatives and conditions for involvement of consumers, retailers and farmers as well as support mechanisms to enable uptake and implementation of our results by the relevant stakeholders and market actors.

Keeping up the momentum now requires the active involvement of many actors. In this context, the main drivers are: citizens, production chains, the European Union and scientists (Blokhuis, 2009).

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ALTERNATIVES TO SURGICAL CASTRATION WITHOUT ANAESTHESIA OR ANALGESIA

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Introduction

About 80 % of the male piglets in the EU and almost all male piglets in Germany are castrated to avoid unpleasant odour and taste of the meat of entire male pigs. According to European law, castration can be performed without anaesthesia during the first 7 days of the piglets´ lives. Various studies however documented painfulness of this procedure. Therefore different research projects have been started to find an alternative to conventional castration of piglets.

Alternative methods have to comply with the following requirements as much as possible:

- **Animal welfare**
  - absent or minimal pain or stress
- **Practicability**
  - little efforts in time and material, realisable by pig farmers
- **Economics**
  - low costs of drugs, equipment, work load
- **Accordance with legislation**
  - animal protection law, pharmaceutical laws, meat-hygiene act
- **Safety**
  - user, environment, prevention of misuse
- **Acceptance**
  - meat quality, residues

Generally there are following potential alternatives to the common surgical castration without anaesthesia for the production of boar meat free of taint:

Procedures without castration:

- ***Raising of entire males*** (slaughter at lower body weight)
- ***Genetic selection*** (breeding pigs with less boar taint)
- ***Immunocastration*** (vaccination against GnRH)
- ***Sperm sorting*** (fattening of female pigs)

Surgical castration with anaesthesia and/or analgesia:

- ***General anaesthesia*** (per injection, inhalation)
- ***Local anaesthesia*** (s.c., i.test., topical)
- ***Analgesia***

Raising entire male pigs have positive impacts on the production efficiency and meat quality due to leaner carcasses and higher protein content, as compared to castrated pigs. Additionally this method improves welfare in early life, but in the advanced fattening period welfare may be impaired due to increased aggressiveness and mutual mounting. Several studies have shown that slaughtering at a lower weight does not entirely eliminate boar taint in all animals. In this context the lack of a rapid and specific on-line detection method in abattoirs for the identification of carcasses with unacceptable levels of boar taint compounds is the major problem.

Boar taint due to high levels of androstenone and skatole is highly heritable. A strictly ***genetic selection*** for low levels of boar taint compounds results in late-maturing pigs and poor fertility. Therefore it is necessary to develop genetic markers to allow the selection of pigs that on the one hand are free of taint from androstenone and on the other hand have good fertility.

Active immunisation against GnRH, so-called ***immunocastration***, with Improvac™ allows the production of male pigs with heavy slaughter weight and improved meat quality, reduced androstenone and skatole levels and reduced aggressive behaviour. Recent studies showed that a small percentage of carcasses had boar taint although the pigs had been correctly vaccinated. Other possible drawbacks are difficulties of vaccinating pigs twice during the finishing phase, accidental self-injection of the operator and perception of the consumers.

A suitable alternative in the future may be ***sorting of sperms*** according to gender and subsequent fattening of only female pigs. Currently this technique is used for scientific approaches. At least several years of research will be necessary to develop an adequate method for commercial routine applications.

Depending on national regulations in several European countries the procedure of anaesthesia is restricted to veterinary surgeons. On that condition a large scale practice of anaesthesia would have enormous logistic and financial consequences. ***General anaesthesia by injection*** induces a sufficient narcosis, but leads to increased piglet losses up to 3-4% and the risk of hypothermia and injury by the sow.

Anaesthesia per inhalation with ***isoflurane*** using a commercial anaesthetic system combined with analgesia (NSAIDs) induces a very effective short-term narcosis which reduces most of the pain and stress caused by castration.
On the contrary inhalation of a ***CO_2/O_2 mixture*** (70%/30%) using also a commercial system failed to a sufficient narcosis in most cases in our own studies. The reduction of consciousness was not sufficient to avoid the sensation of pain. During induction of CO_2-anaesthesia strong reactions of discomfort such as restlessness and hyperventilation as well as severe disturbances of the cardiovascular system were observed.
Local anaesthesia with ***injection of lidocaine or procaine*** into the testis or spermatic cord is effective in reducing acute pain during castration; adverse effects are the induction of pain by the injection itself, little or no effect to stress parameters, no alleviation to long-term pain due to castration and wound-healing disorders in several cases. ***Topical anaesthetic*** techniques showed some anaesthetic effects on the skin but overall were much less effective than the local anaesthesia per injection.
The preoperative administration of ***analgesics*****,** which is not restricted to veterinarians, leads to a reduction but not to an elimination of pain and stress response during castration. In some studies a reduction of postoperative pain has been demonstrated.

In summary each alternative to the common surgical castration without anaesthesia has assets and drawbacks. In addition there is a need for further research to optimize new approaches until the castration of male piglets in general can be replaced.

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WELFARE, BEHAVIOUR AND PAIN

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Animal welfare can be defined in a number of different ways, but there is a growing consensus that whatever the definition, it has to include three elements: the emotional state of the animal, its biological functioning and its ability to show normal patterns of behaviour (Duncan and Fraser, 1997; Mendl, 2001). Since the subjective feelings of the animal are an essential part of its welfare (Dawkins, 1990) a logical argument is that welfare will be reduced by negative subjective states such as pain, for example.

The possibility to express normal patterns of behaviour has long been recognized as an important element of good welfare. Recently, the Welfare Quality® project recommended an animal welfare assessment system that includes the expression of social and other behaviours (Botreau et al, 2007).

Pain in pigs may be a consequence of disease, injuries, some husbandry procedures such as castration and tail docking, and farrowing.

Absence of disease is a basic requisite for good welfare, as diseases can cause pain and may interfere with normal behaviour. Chronic diseases often have a debilitating effect on the animal and may lead to it being culled. Some diseases that are more relevant from an animal welfare standpoint are called “multifactorial diseases”, meaning that they are caused by the interplay of several factors

Injuries resulting from accidents, poor flooring and inadequate design or maintenance of housing facilities, fighting with other animals, tail-biting and feather pecking can also cause acute and chronic pain.

Negative social interactions, such as aggression, clearly impair animal welfare. Aggression may not only result in injuries, pain and, in extreme cases, the death of the animal, but it also leads to fear and stress within the whole group (Fraser and Rushen, 1987). Fear is an aversive emotional state and, although fear behaviour can be adaptive in ideal circumstances, its sudden, intense or prolonged elicitation (and the consequences thereof) is a major welfare problem (Jones, 1997). Stress can harm body functioning by impairing immune function and reproductive performance, and decreasing food intake. Also, negative social interactions may interfere with the expression of normal behaviour, particularly in low ranking animals, and thereby reduce food intake and resting time which may in turn lead to debilitation and health problems, such as lameness.

Fighting in pigs is more common when animals are mixed with unacquainted individuals and when animals have to compete for access to feed, water or resting space. Social disruption may also lead to a reduction in positive social interactions and to lowered production. Mixing of unfamiliar animals (often with a change of physical environment) is a common practice in pig husbandry particularly at weaning, at the beginning of the growing-finishing period and during transport to slaughter. Mixing-induced aggression mainly reflects pigs fighting in order to establish dominance relationships, most aggressive interactions being typically shown during the first few hours after grouping. The frequency of fighting then steadily decreases to a very low level by 24-48 h post-grouping, when the hierarchy becomes fairly stable (Meese and Ewank, 1972).

Housing conditions that result in increased competition for resources can also heighten the number of negative social interactions. This may happen if stocking density is too high or when access to resources is insufficient, for example when feeding space is limited. Ewbank and Bryant (1969) concluded that high stocking rates cause the dominance hierarchy to be less successful in controlling aggression within the group, thereby increasing the incidence of agonistic behaviour. High stocking densities increase aggression because the easy escape of attacked individuals is

thwarted. In cattle, for example, reduction of feeding space increases competition for feed and this in turn increases aggression.

Tail-biting is an important cause of injuries in pigs. According to the most widely accepted hypothesis, tail biting is a form of redirected behaviour derived from the thwarting of normal exploratory, feeding, social and sexual motivations. Animals are strongly motivated to perform particular behaviour patterns. This is the case, for example, with rooting in pigs, nest building in sows and hens, ground pecking and scratching in poultry, and exploration in all species. In some circumstances, the inability to perform such behaviour patterns may cause distress and lead to the development of damaging behaviours. For example, tail biting in pigs and feather pecking in poultry may reflect the lack of opportunity to perform rooting and ground pecking / scratching, respectively.

Tail-biting is a welfare problem because of the pain and suffering experienced by the bitten animal (not only due to the biting but also to secondary infections), the stress caused to the group (restlessness), and the likely frustration of the biting animal (Scientific Veterinary Committee, 1997). As is true for other behaviour problems in intensive pig production, tail biting is a multi-factorial problem involving both internal and environmental risk factors; these include genetic background, sex, age, health status, diet, feeding management and different characteristics of the pen (Moinard et al, 2003). Feather pecking in chickens and other poultry species has similar deleterious effects.

Several procedures that are routinely carried out in farm animals can cause pain. In pigs, these include tail docking, castration and tooth clipping. The pain associated with these procedures normally lasts a few days, but in some cases chronic pain may also result. Though these management procedures are often carried out on young animals they too can feel pain. Some of these procedures are in theory carried out to prevent other, potentially more severe welfare problems. For example, tail-docking is intended to prevent tail-biting while beak trimming is used to minimise feather pecking/cannibalism. However, since both are multifactorial problems, tail-biting and feather pecking should be addressed by improving housing conditions, providing environmental enrichment, and perhaps selective breeding rather than by routine tail-docking or beak trimming.

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ORGANIC PIG HUSBANDRY SYSTEMS

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Introduction

Consumers are becoming increasingly interested in foods produced according to ethical aspects of animal health and welfare principles. Organic farming is often directly associated with an enhanced level of animal health and welfare, and many consumers conflate organic and animal-friendly products (McEachern and Willock 2004). The EU-Regulation (EEC-No. 1804/1999) on organic livestock production, now replaced by EEC-No 834/2007, was introduced to provide a framework ensuring living conditions for organic livestock to be better than those in conventional systems, to harmonize the rules across member states, and to make all organic systems across EU members subject to minimum standards. Scientifically based information on how and to what degree the EEC-Regulation contributes to the objective of a high status of pig health in organic farming is scarce. In the following, the current state of the art is presented from an European perspective.

Living conditions for organic pigs

According to the framework of the EEC-Regulation, a land based production, including the use of a high proportion of home-grown feed, is a key feature required from organic livestock systems. Roughage must be offered at all stages of the production on a daily basis. Inclusion of synthetic amino acids in pig rations is banned. Minimum weaning age for piglets is 40 days. The standards require access to an outdoor area for all animals. Entirely slatted floors are forbidden.

Animal health and welfare of livestock in organic agriculture should be promoted primarily by preventive measures using appropriate breeds, feeds and feeding practices and husbandry techniques. Furthermore, prophylactic use of drugs is prohibited and restrictions are placed on conventional medicine use.

The minimum standards in organic livestock farming clearly exceed the legal minimum requirements of conventional livestock production in many areas. Hence, the improved living conditions are likely to have beneficial impacts on the health and welfare of pigs, but this production framework poses also various challenges for the farm management.

Although defined by specific and basic guidelines that are valid throughout Europe, organic pig husbandry systems vary to a high degree between countries, depending on the prevalent conventional systems. In Western Europe and in Scandinavia, more intensive systems have been converted to organic production by running the breeding stock outside during lactation and allowing pigs after weaning access to outdoor pens. In Austria and Germany, organic pig production is primarily limited to small units where pigs are kept indoors with access to an open yard, whereas outdoor rearing of organic slaughter pigs in Sweden is a standard production method.

Largely heterogeneous farming conditions allow for huge differences in the implementation of feeding regimes, preventive and hygiene measures and the use of remedies etc., all of which variously impact animal health and welfare. Detailed on-farm assessment in Germany brought up weak points in hygienic, nutritional and animal health management (Dietze et al. 2007). Measures considered to be standard in conventional farming such as electronic livestock data acquisition, barn disinfection, regular feedstuff analysis or effective disease prevention measures were not implemented consequently on all organic pig farms. Furthermore, the farms showed huge differences with regard to the availability of relevant resources such as labour time, feedstuffs of high quality or investments.

Impacts on animal health

There is a growing body of epidemiological evidence on the impact of organic management on the health and welfare of livestock (Hovi et al. 2003). Data from various surveys suggest that, whilst the difference in production systems and national disease situation significantly affect the health and welfare problems experienced in organic pig production, endo- and ectoparasites appear to be the most common concern for organic pig producers when compared to conventional production (Vaarst et al. 2000; Vermeer *et al*. 2000; Dietze et al. 2007). On the other hand, the prevalence of respiratory diseases in fattening pigs was markedly reduced in organic compared to conventional herds (Baumgartner et al. 2003).

Due to the complex interactions among farm animals, microbes and living conditions, the identification of the specific causal factors

responsible for the corresponding production disease on each farm is a big challenge for veterinarians. Correspondingly, there is substantial variation in the herd status in relation to animal health and welfare.

Analysis of the literature reveals that the average prevalence of production diseases in conventional and organic livestock production is comparable high and at the same time varies widely between farms and between countries (Sundrum et al. 2004). Differences between farming enterprises can be considered higher than differences between the production methods. Thus, the current approach of organic livestock production to ensure animal health by upgraded minimum standards lacks efficiency in the implement-tation of organic principles (Sundrum et al. 2006).

Conclusions

The fact that animal health situation does not appear to be consistently better on organic than on conventional farms raises questions on the impacts of management and systems changes. Obviously, organic guidelines play a minor role with respect to the status of animal health and welfare, whereas differences in management practices and control tools within the farm system might be the main reasons for substantial variation in the prevalence rates of diseases between farms.

It is concluded that any improvements in relation to animal health and welfare should be based on the implementation of monitoring systems including clinical assessments, abattoir data and review of treatment data, as well as health planning strategies taking into account feedback mechanisms. The latter, however, require clear guidelines concerning the level of production diseases expected as the output of the farm system. Currently, there is no common agreement about a threshold acceptable for organic livestock farming or about categories that allow to group different animal health levels in a range between very good and worse. Consequently, there is a need for a change in the paradigm from standard and resource oriented to a result and outcome oriented approach.

The current animal health status on many organic farms reveals severe discrepancies between the claim and reality with regard to organic products. There is a need to develop on-farm control measures and assessment tools with regard to the animal health status to meet the expectations of many consumers. Certification bodies should establish a regular monitoring system for animal health data. Producers failing to meet certain health standards in the longer term should face consequences, such as imposition of improvements schemes and loss of product certification.

The previous results indicate that any improvements and increased levels of minimum standards, e.g. as a part of legislative regulations or as brand label programmes cannot be expected to represent an appropriate tool to predict and improve the level of animal health and welfare of farm animals.

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MANAGING THE HIGH PRODUCING SOW DURING FARROWING, LACTATION AND OESTRUS

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Introduction

The highly prolific sow, defined as one giving birth to 16 or more liveborn piglets, presents a challenge to reproductive management of a sow herd (Boulot et al., 2008). From the point of view of reproductive management of the sow, feeding plays a key role. Therefore, recent findings related to feeding and how it affects farrowing, lacation and oestrus management are given the highest priority in the present paper. We report on recent findings related to feeding sows a high fiber diet during the period preceding parturition and its beneficial effect on gut function and duration of farrowing (Oliviero et al., 2009). In addition to feeding, arrangement of the farrowing pen (crate vs. pen; barren vs. enriched) appears as a critical factor determining the course of parturition. Our latest findings suggest that prohibiting the sow to exhibit nest building behaviour (Algers and Uvnäs – Moberg, 2007) prolongs parturition by an average of 90 minutes (Oliviero et al., 2008; 2009).

In addition, feeding sows with a high fiber diet during pregnancy, apart from being a beneficial feeding strategy from the welfare point of view, appears to increase the ad libitum feed intake during lactation. This effect seems to be carried over to the average daily gain of piglets, especially during the neonatal period (Quesnel et al., 2009, Peltoniemi et al., 2009). Amount of feed eaten by sows during lactation, on the other hand, appears as a key in enhancing gonadotrophin secretion and follicle development throughout lactation, however these effects of feeding become more evident towards the end of lactation (Kauffold et al., 2008). Follicle growth after weaning, as triggered by gonadotrophins FSH and LH, occurs the faster the better the stimulation by gonadotrophins has been prior to weaning (Prunier and Quesnel, 2000; Kauffold et al., 2008).

Succesful farrowing

Succesful farrowing can be defined as (1) sows given a chance to exhibit species specific nest building behaviour, (2) duration of farrowing not exceeding 5 hours, (3) all piglets in the litter born alive and (4) the first sucklings resulting in all newborn piglets receiving colostrum.

As fetuses grow fast at the end of pregnancy, there is a need to increase the energy intake by the sow. A common feeding strategy has been to put sows on lactation diet during the period before farrowing. While being understandable as a strategy from the energy intake point of view, this strategy by large ignores the intestinal function during and shortly after farrowing. We observed that after having been on a traditional lactation diet with 3,8 % of crude fiber prior to farrowing, sows showed an increased constipation incidence of up to 20 % for several days after farrowing (Oliviero et al., 2009). Gut function slows down during parturition anyway and overgrowth of bacteria in a gut filled with an energy-rich diet may lead into activation of the GALT (gut associated lymphoid tissue) system (Oliviero et al, 2009; Reiner et al., 2009). The activation of the GALT system would stimulate PGE_2 release, which may further suppress intestinal function until a leak of endotoxins through the gut wall occurs, bringing about a systemic response and clinical symptoms found in post partum dysgalactia syndrome, PPDS (Reiner et al., 2009). Therefore, avoiding constipation by any means should be of interest to managers of any well functioning piglet producing unit.

While feeding sows laxatives like Glauber salt, cooked linseed mixture and commercial laxatives are among traditional measures to avoid constipation, our experiences support adding more fiber into sow diets before farrowing (Oliviero et al., 2009). A 7-11 % crude fiber content prior to farrowing appears as a reasonable measure to prevent constipation and an important part of management of successful farrowing (Oliviero et al., 2009; Quesnel et al., 2009; Peltoniemi et al., 2009). The first beneficial function of a high fiber diet is the improved intestinal activity (Oliviero et al., 2009). However, other beneficial effects relating to the use of high fiber diets have also been reported. These include improved intestinal immunity due to an increased mucin production as well as improved energy utilization of the feed consumed.

Water intake of the sow before farrowing is an important parameter to monitor, since it is elementary to milk production. On average, sows drink 10-30 liters of water around farrowing (Oliviero et al., 2009). However, variation between individual sows was considerably large and sows on the high fiber diet drank significantly more than did sows on the traditional diet. This was attributed to either stimulating effect of fiber on water intake as such or the increased volume related to the higher fiber diet possibly explaining the difference compared to the traditional pre-farrowing diet (Oliviero et al., 2009). Whatever the cause, these findings encourage use of diets containing more fiber prior to farrowing. The usual flow recommendation of drinking water from nipples is 3-4 liters / minute and this is one of the easiest parameters to me measured on a health check call in a farrowing unit.

Data from our group show that sows not given a chance to express nest building behaviour have higher circulating cortisol concentrations before farrowing and lower oxytocin concentrations during the expulsion phase of farrowing (Figure 1; Oliviero et al., 2008a). From the practical viewpoint, however, maybe the most important observation was the one related to the duration of farrowing. If not given a chance to build up a nest in a crate – free pen, it took an average of 1,5 hours longer from our experinmental sows to deliver the litter (Figure 1; Oliviero et a., 2008a; 2009). Furthermore, the interval between piglets was considerably longer in crated sows with no nest building material (25 minutes CRATE vs. 16 minutes in PEN; Oliviero et al., 2008a).

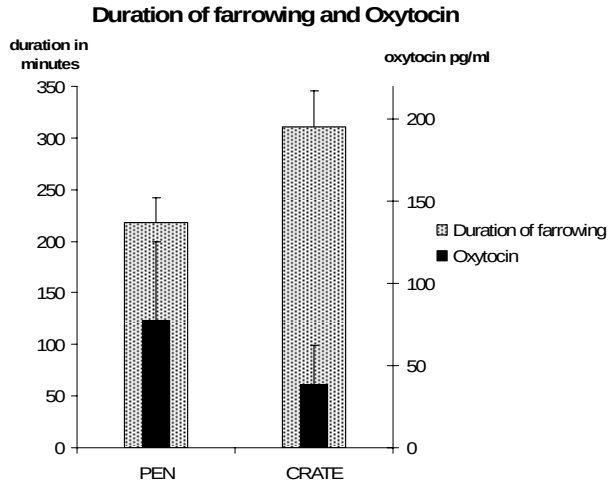

Figure 1. Average duration of farrowing and average Oxytocin post-expulsion pulses in the PEN (n = 9) and CRATE (n = 9) groups of sows (mean ± SD). Data from Oliviero et al., 2008.

We have recorded duration of farrowing in several studies by now (Oliviero et al., 2010). From these studies it is apparent that a farrowing lasting longer than 5 hours is deemed unsuccessful and easily leads to complications for both the dam and the newborn. Therefore, we suggest that with the modern sow lines, a 5 hours threshold may be applied when making a difference between successful and unsuccessful farrowing. Several factors, as constipation, body condition of the sow, parity, breed and number of stillborn piglets affect duration of farrowing (Oliviero et al., 2010). Constipation, as discussed above, may be alleviated by increasing fiber content of the diet. Backfat thickness of higher than 17 mm, however, also appeared as a risk factor for prolonged farrowing in the genetically lean Finnish sow population (Oliviero et al., 2010).

Number of piglets born still prolonged duration of farrowing (Oliviero et al., 2010). It is known from previous studies that fetuses are usually alive shortly before farrowing. The great majority (>80%) of piglets that are deemed to born still, die during the course of parturition. It is therefore reasonable to assume that dystocia, whether due to the dam or the fetus, is the major cause for piglets born still. According to Jackson (1972), in the sow, inertia uteri (37%) used to be considered as the most common cause for dystocia followed by the breech presentation (14,5%), obstruction of the birth canal (13%), simultaneous presentation (10%), downward torsion of the uterus (9%), downward deviation of the head (4%) and fetal oversize (3,5%). However, these robust classifications do not account for the more precise causes for intrapartum deaths of piglets, such as strangulation of the umbilical cord or considerations relating to uterine contractions.

It is very well known, that piglets need their passive immunity acquired through colostrum, otherwise they will be in big trouble. As every piglet is in need of colostrum and one that is missing out is clearly

the one at risk of loosing life already in the early days. Colostrum transmission through the gut wall of the piglet can only occur during the first day of life and the amount of colostrum is not increasing according to the number of piglets born. Therefore, in a large litter, supervision is required, not only during the process of parturition, but also attending that even the last piglets born will receive adequate amount of colostrum.

Lactation performance of the sow and piglets

To care for a large litter, adequate feed intake of the sow is of great significance. A drop in feed intake around farrowing can be considered as physiological. Stepwise rise in feed intake by 0,5 kg of feed / day until day 10 or so will be enough to reach the target daily intake of about 8 kg, equal to about 100 MJ ME for a sow nursing 12 piglets (Peltoniemi and Kemp., 2009). Too steep a rise in feed intake after farrowing will jeopardize the success of the whole lactation, since there will be a decline in intake, followed by fluctuation of it for most of the duration of lactation.

A balanced nutritional program designed to avoid excessive weight loss during lactation is of great importance for successful lactation. It has been shown that feeding the sow with higher amounts of fiber during pregnancy, intake of feed during lactation can be increased with a corresponding increase in performance of piglets (Quesnel et al., 2009). It has also been shown, that increasing fiber intake throught the reproductive life of the sow appears as a feasible approach, contributing to improved welfare of the sow as well as good reproductive performance of the sow and improved growth performance of piglets (Peltoniemi et al., 2009).

Piglet mortality and mean birth weigh appear to be closely associated with littersize. When litter size increased from 10 to 15, number of stillborn piglets went up from 0,3 to 1,0 and the proportion of piglets weighing less than 1 kg went up from 3 to 15 %, respectively (Boulot et al., 2008). In large litters, supervision of farrowing is therefore necessary. The first issue there is an accurate prediction of farrowing. We have developed techniques, whereby prediction of farrowing becomes possible and feasible. Photosensors monitoring impending farrowing can be used to predict the expulsion phase of farrowing 24 hours prior to the first birth of the first piglet (Oliviero et al., 2008b). Other modern technology, such as use of thermocameras may be applied to detect hypothermic newborn piglets that require immediate attention by the caretakers.

We argue that the present development regarding piglets born in the pig industry is not on a sustainable basis. Instead of the number of piglets born alive, more research effort should be aimed at increasing the birth weight of piglets born and decreasing the still birth rate. Moreover, more attention should be paid to the quality of newborn piglets, the quality of piglets weaned and the quality of fattening pigs. The quality of piglets and fattening pigs in the pig production may be achieved by long term studies, starting during the fetal period, focusing on early development of the piglet and finally exploring the fattening phase of the pig.

Gonadotrophins and follicle development

Intake of feed by sows during lactation appears as a key in enhancing gonadotrophin secretion and follicle development throughout lactation, however these effects of feeding become more evident towards the end of lactation (Kauffold et al., 2008). Follicle growth after weaning, as triggered by gondatrophins FSH and LH, then occurs the faster the better the stimulation by gonadotrophins has been prior to weaning (Kauffold et al., 2008). Decreasing lactation length is a commonly taken strategy in Europe to hasten the reproductive cycle of the high producing sow and avoid excessive weight loss. However, the sustainability and ethical grounds for these strategies need thorough attention in the near future.

After weaning follicles grow approximately 1 mm / day. They reach the ovulatory size at about 7-10 mm within a week after weaning. The most recent findings in our group suggest that follicles ovulating at the size of 7 or 8 mm result in improved fertilization rates and larger litters than follicles ovulating at < 7 mm or > 8 mm (Vehmas et al., unpublished). Ultrasound technology (US) can nowadays be effectively applied in mating units to monitor insemination accuracy of insemination operators. In problem farms, inseminations occuring too early or too late in relation to ovulation can be picked up by US and relevant recommendations to change the AI strategy can be given. The optimal insemination time is estimated to be 0-16 hours prior to ovulation, which occurs at approximately when two thirds of the standing oestrus has passed.

Follicle development can also be monitored with regard to possible not-expected physiological or pathological findings at the ovary. After weaning, detection of corpora lutea may indicate lactational oestrus, while cystic follicles, for instance, are among classical examples of application of ultrasound technology in sow herds.

Conclusions

Succesful farrowing includes components of maternal behavior, duration of farrowing, piglet mortality and colostrum intake. Feeding is considered as the major factor in the reproductive management of the hyperprolific sow. New insights such as adding more fiber to sow diets during pregnancy and especially in the period prior to farrowing prevent constipation, increase water intake of the sow around parturition and increase milk intake and performance of piglets. Use of modern technology in supervision of farrowing may improve losses related to large litters. Use of ultrasound technology after weaning to monitor follicular growth may further improve littersize. In breeding programs, new components of maternal characteristics such as maternal behavior, ease of parturition, colostrum production, and piglet quality parameters may be taken to further improve success rate of reproductive management.

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USE OF ULTRASONOGRAPHY FOR REPRODUCTIVE TROUBLESHOOTING

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Over the last 15 years B-mode ultrasound has been accepted more and more by the pig industry as a valuable tool in the management of sow/gilt reproduction (1). This has also been possible through intensive research demonstrating that by using ultrasound the female reproductive tract can be made visible, which then allows for the delineation of normal (healthy) from abnormal (diseased) genital conditions. It is also argued that the fact that ultrasound can be done transcutaneously versus transrectally, most likely facilitated a broad acceptance, since this route of application is the least laborious and also the safer approach. Ultrasound is currently used for pregnancy diagnosis as well as for uterus and ovary scanning, and may then be utilized in order to check for ovulation and puberty as well as for scanning of reproductively diseased females. It is also worth mentioning here that B-mode ultrasound can be used for backfat measurement and for evaluation of the urinary bladder, as well as the reproductive organs of boars including testes and epidydimides (2,3).

“Troubleshooting” in reproduction, means dealing with herd issues rather than with individual reproductively failed animals. Each troubleshooting case is unique. However, they may share similarities with each other. “Troubleshooting” always means to start a work-up cascade which depends on the case and comprises more or less of diagnostic steps. Identifying the problem based on analysis of records is always the first task that has to be done and is followed or accompanied by discussing the problem with the producer and/or veterinarian in order to gather additional information. Once the problem is identified, a diagnostic plan has to be developed. While developing this plan the diagnostician will consider possible causes for the reproductive failure (“differential diagnosis”) and available diagnostic procedures to address the differentials. Also, the cost-benefit equation needs to be brought in.

Typical examples for reproductive failure at a herd level that the authors have encountered during the past 15 years, include i) gilts not coming into heat; ii) no heat after weaning; iii) silent heat; iv) low conception rate (i.e. low pregnancy rate as determined by pregnancy check within the first month after breeding); v) low farrowing rate; vi) high number of late term fall-outs (pregnancy check positive, but no farrowings); vii) small litters. Any of those cases may have either a truly biological cause (i.e. sow and/or semen/boar failure), or is the result of management failure; the latter still being the number 1 reason for reproductive failure in female swine. Thus, any diagnostic work-up has to include management evaluation as well as the animals. It is, however, often the lack of any “biological causes” that uncover management issues as the real reason for the reproductive failure.

For no heat or silent heat, whether in gilts or weaned sows, evaluating heat detection procedures and the personnel doing heat detection is a good starting point and may then give some indication as to whether heats were missed because of inadequate heat detection procedures. However, since the phenomenon of no/silent heat is essentially related to the ovaries, only the visualization of the ovaries and determination of ovarian bodies using ultrasonography brings final proof. In cases of low conception or farrowing rate, it is just logical to start the diagnostic work-up with checking ovulation in order to determine when animals ovulate relative to breeding. If a discrepancy can be excluded, then checking for early embryonic losses by ultrasonography may follow. It is imperative to mention, that in case of low farrowing rate and/or a high rate of not-in-pigs (NIP), procedures used for pregnancy checking need to be carefully evaluated too. While it is known that the return-to-service checks, which is basically heat detection, is usually the least reliable procedure among those used for pregnancy testing, but other procedures such as ultrasonography can also fail. As an example, the author has been involved in a case of presumed late term fall-outs where at the end (after months of diagnostic work-up) the person who did the scanning together with a poor ultrasound unit was identified as the source of the “failure” (i.e. open animals were misdiagnosed as pregnant and ended up as late fall-outs). Another advantage of using ultrasonography as a core diagnostic tool is that, based on the findings, additional diagnostics procedures such as pathomorphology of genital tracts or mycotoxicologic examinations may then be better directed. This systematic approach of troubleshooting with ultrasonography as the diagnostic corner stone has been found very successful in solving a multitude of reproductive problems.

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THE SECOND LITTER SYNDROME IN SOWS

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Introduction

Reduced litter sizes in 2nd parity sows are often seen and are referred to as the Second Litter Syndrome (e.g. Morrow et al., 1992). The causes of the reduced litter sizes of especially 2nd parity sows seem to lie in the body weight loss during preceding lactation (reviewed by e.g. Prunier et al., 2003; Thaker and Bilkei, 2005). Animal experiments in which 1st parity sows were fed restricted during lactation have shown that follicle development during and after lactation is compromised (e.g. Quesnel et al., 1995), ultimately resulting in a lower embryo survival (e.g. Zak et al., 1997). This lower embryo survival may not only result in lower litter sizes, but also in lower farrowing rates.

In a current PhD-project concerning the Second Litter Syndrome, we studied the influence of the sow body weight development during 1st parity on 2nd parity reproductive performance and, in a second analyses, we studied whether 2nd parity reproductive performance is related with reproductive performance in subsequent parities. These two questions were studied by analyses of farm data.

Results and Discussion

Sow development

Data from two experimental farms (A and B) of Wageningen University and Research Centre (1999-2005; 250 sows in farm A and 270 sows in farm B) were analysed to study whether sow development parameters affect the reproductive performance (litter size from 1st insemination and prevalence of rebreeding) of 2nd parity sows. Litter sizes in 1st and 2nd parity were, respectively, 10.7±0.1 and 11.6±0.2 for farm A and 11.8±0.1 and 11.6±0.1 for farm B and the prevalence of reduced litter size was 31% on farm A and 45% on farm B. The prevalence of rebreeders in 2nd parity was 11% for farm A and 15% for farm B.

The farms also strongly differed in the weight development of their sows. Compared with gilts from farm B, gilts from farm A were younger and lighter at first insemination (230±0.6 days and 124±0.5 kg for farm

A vs 275±0.9 days and 145±0.8 kg for farm B), first farrowing (resp. 181±0.9 kg vs 189±1.1) and first weaning (resp.156±0.9 kg vs 165±1.1). Weight loss during pregnancy was similar for both farms (resp. 23.7±1.0 kg vs 24.9±0.7). Gilts from farm A, however, gained more weight in the period between first insemination and first weaning compared with gilts from farm B (resp. 36.1±0.8 and 20.9±1.3 kg).

Also the variables associated with litter size and repeat breeding in 2nd parity differed between the two farms. On farm A, where the sows were younger and lighter at insemination, mainly sow weight parameters were associated with litter size and rebreeding in 2nd parity, whilst on farm B litter size in 1st parity was associated with litter size and rebreeding in 2nd parity. On both farms, however, not only lower lactation weight loss, but also a higher weight gain from first insemination to first weaning was associated with a decreased risk of rebreeding (Odds Ratio 0.7 per 10 kg for farm A and 0.8 per 10 kg for Farm B) and on farm A also with a lower litter size in 2nd parity (β=0.42 per 10 kg weight gain).

These results show that sow live weight development from 1st insemination onwards affects reproductive performance in 2nd parity, especially when gilts are relatively light and young at first insemination.

Subsequent performance

Data from 87 Dutch sow farms (2000-2008; 47,000 sows) were analysed to study relations of litter size from 1st insemination and from rebreeders in 2nd parity sows reproduction in subsequent parities. Litter size in 2nd parity was was divided into three classes: 2L: litter size ≤10 piglets, 2M: litter size 11 to 13 piglers and 2H: litter size ≥14 piglets. The summed litter sizes of 3rd to 5th parity increased with an increase in 2nd litter size, from 35.7±0.3 (2L) to 36.9±0.3 (2M) to 39.6±0.3 (2H). Litter size in 2nd parity was not related with the prevalence of rebreeding in subsequent parities.

Farrowing rate from 1st insemination was lower in 2nd parity sows (79.9%) compared to 1st parity sows (81.2%) and older parity sows (85.2%). Repeat breeders in 2nd parity also had a lower farrowing rate in 3rd parity (-4.1%) and in 4th parity (-3.4%). Rebreeding in 2nd parity was not related with litter size in subsequent parities.

A reduced performance in 2nd parity sows (both litter size and rebreeders) was also associated with a higher culling rate.
Thus, sows with a lower reproductive performance in 2nd parity, on average also have a lower reproductive performance in subsequent parities. It is interesting to note that the phenomena of rebreeding on the one hand and litter size on the other hand do not seem to be associated in this respect, since rebreeding in 2nd parity was only related with the chance of rebreeding in subsequent parities and litter size in 2nd parity was only associated with litter size in subsequent parities. At the moment, it is not clear what causes these relations with subsequent performance, further analyses are being performed.

Conclusions

Besides reduction in litter size in 2nd parity, the second litter syndrome is also manifested in a higher chance of rebreeding of 2nd parity sows. Both reproductive parameters are influenced by gilt and sow body development up to weaning the first litter and thereby influence the severity of the second litter syndrome on a farm. A reduced reproductive performance in 2nd parity is associated with reproductive performance, both litter size and the prevalence of rebreeding, in subsequent parities. As a consequence, also the culling rate is affected.
Further studies will be conducted to unravel causes and consequences of the 2nd parity performance and possibilities to decrease its impact.

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*The results presented come from ongoing analyses in the PhD-project of Lia Hoving

OPTIMIZING INSEMINATION

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Introduction

Amongst factors influencing fertility in pig herds, the management of artificial insemination (AI) is frequently determinative. On a number of farms subfertility is not obvious, nonetheless, there could be better use of the fertility potential. Estrus control, the timing and number of inseminations, the technique of AI, semen storage on farm and the use of new AI technologies, all require a specialised knowledge of pig reproductive physiology. Veterinarians have to analyze individual farm potential for enhancement of fertility, possible causes of subfertility, and need to develop farm-specific strategies to improve insemination management. The present paper reviews the current status for optimizing insemination on pig farms beyond the background of reproductive physiology.

Boar stimuli

Correct timing of insemination requires careful detection of the onset and end of oestrus at correct intervals. Boar stimuli are important in promoting follicular development and expression of oestrous behaviour in sows (Langendijk et al. 2006). Additionally, a high level of boar stimuli increases the frequency of uterine contractions, indicating a supportive role for passive sperm transport through the long uterine horns at the time of insemination. Noteworthy, this effect cannot be sufficiently mimicked by a robot teaser boar which emits olfactory, acoustic and visual boar cues (Gerritsen et al. 2005). Increase of oxytocin concentrations in peripheral blood plasma occurs in immediate response to boar presence and lasts for approximately 10 min (Langendijk et al. 2003). Therefore, exposure of sows to the boar during both back pressure testing and insemination is crucial.

Timing of insemination

In the Nineties, studies related to time-relationships of oestrus, ovulation, insemination and fertilization success were done worldwide using ultrasound for the detection of ovulation. The results essentially remain valid today, with the key observation that ovulation occurs at the beginning of the last third of oestrus regardless of the overall duration of oestrus. Precise prediction of the time of spontaneous ovulation in individual pigs has not yet been achieved; however, prediction of oestrus length by observation of the onset of oestrus after weaning has found broad acceptance in AI practice for calculation of the expected time of ovulation (Weitze et al. 1994). With respect to sperm survival in the female tract, insemination should be timed as close as possible to ovulation, at a maximum within 12 to 24 h before ovulation. The benefit of sonographic diagnosis of ovarian morphology for pig fertility management in practice has been shown (Große Kock 2004). Determination of the time of ovulation in relation to oestrous behaviour and AI mangemanement in representative numbers of sows on consecutive days has a great potential to provide short cuts in AI timing and to develop farm-specific stragegies for improvement of AI management.

Semen storage

In Germany, most AI centres guarantee the potential for semen use up to 72 h of storage without compromising fertility. At the same time, the world-wide AI industry is developing "long-term" boar semen extenders promising preservation lengths of up to 15 days. Indeed, the potential for long-term storage would significantly increase the flexibility for semen production, transport and storage on farm. Individual boars differ in their capacity for semen storage regardless of the extender used. Commonly, boar sperm membranes are highly susceptible to ageing and temperature imbalances. Such storage effects are often not visible at first in loss of motility, but nevertheless they may influence sperm function after a few days of storage, as can be shown by recently-developed advanced sperm diagnostics (Henning et al. 2009). For semen storage on farm, temperature must be held between 15-18°C without exceeding the recommended storage length.

Use of new AI technologies

The development of techniques to inseminate with low numbers of sperm in a small volume would increase insemination efficiency when using spermatozoa of high value but otherwise impaired, e.g. by freezing and thawing or sex-sorting. Post-cervical or intrauterine insemin-

ation with several devices has been developed to traverse the cervix and deposit sperm in the uterine body or posterior horn of multiparous sows. Compared to standard transcervical AI, post-cervical AI allows a threefold reduction in numbers of spermatozoa to be inseminated, whereras deep intrauterine AI allows a 5 to 20 fold reduction (Vazquez et al. 2008). The use of post-cervical insemination varies among and within countries. Limits may arise from the use in sows only, skills needed for catheter handling, and the possibility of damaging cervical or uterine tissue. Laparoscopy offers the possibility of inseminating a very low number of spermatozoa (i.e. 0.3×10^6) into the oviduct in anaesthetized pigs. However, the risk of polyspermic fertilization is substantial. Due to surgical intervention, its use is not appropriate in practice.

Conclusions

A number of AI management factors influence the chance of spermatozoa meeting the oocytes at the stage of their full fertilizing capacity. Among those, estrus detection and timing of insemination have a high impact on fertilization results on pig farms. Based on a deeper knowledge of reproductive physiology and of modern diagnostic strategies, veterinarians have the realistic potential for optimizing insemination on pig farms.

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BRACHYSPIRA HYODYSENTERIAE CLONES DISSEMINED WITHIN DIFFERENT EUROPEAN COUNTRIES

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Introduction and Objectives

Swine dysentery (SD) is a severe mucohaemorrhagic diarrhoeal disease caused by infection with the intestinal spirochaete *Brachyspira hyodysenteriae*. SD occurs worldwide, and in recent years has caused significant problems in many European countries. The research group at León University has identified *B. hyodysenteriae* infection in pigs throughout Spain (1), and has recently characterised these using multilocus sequence typing (MLST) (2), in collaboration with Australian workers. The purpose of the current study was to use MLST (3) to determine whether clonal groups present in Spain are also disseminated in other European countries.

Materials and Methods

Sequence data from 16 European isolates of *B. hyodysenteriae* previously analysed by MLST (3) were obtained from PubMLST and were compared with the results obtain for 50 Spanish isolates of *B. hyodysenteriae*, as previously described (2). Seven MLST loci were used and isolates were considered genetically identical and hence of the same sequence type (ST) if they were identical at all seven loci.

Results

The predominant ST previously identified in Spain (ST1) was shared by a British tiamulin resistant isolate, E2 (4). In a previous study, E2 and six isolates from several farms with outbreaks of SD in the South-East of England showed the same PFGE pattern, indicating that they belonged to a single clone (4). At least four of the farms were connected through pig movements (5), and five of the isolates were tiamulin resistant. ST1 also was widespread in Spain, and several of the 18 Spanish farms where it was identified had connections. Most of these isolates were recovered from white commercial pigs, but five were from Iberian pigs, a local rustic breed traditionally reared in extensive units. ST2 (2) included 7 isolates from 7 farms located in four different autonomous regions in northern Spain (Fig. 2). This genotype previously was described for two German indole-negative isolates, one of them tiamulin resistant, and isolate Be45 from Belgium (3). At least 3 of the Spanish ST2 isolates also were indole-negative (6). ST2 was widespread in Spain, but it was not recovered from Iberian pigs. Representatives of the other European STs were not identified in Spain.

Fig. 1. Map representing in yellow the 8 autonomous regions of Spain where ST1 (described for the first time in south-east England) were detected.

Fig. 2. Map representing in blue the 4 autonomous regions of Spain where ST2 (described for the first time in north-west Germany and Belgium) were detected.

Discussion

This study has provided an insight into the origin and dissemination of *B. hyodysenteriae* isolates between European countries, using a reliable method that provides useful molecular epidemiological data. In the case of two common STs, there was evidence of likely transmission of strains between farms, and between countries in Europe. It is known that there is trade of pigs between European countries and Spain (source: Spanish Ministry of Environment and Rural Affairs). The real extent to which ST1 has spread since 1998 is not known, and should be studied. Considering that tiamulin is one of the few drugs used for treatment of SD in Spain, as in many other countries, this clone and other tiamulin resistant clones are likely to be selected for and spread further.

Acknowledgements

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SOUND ANALYSIS FOR HEALTH MONITORING IN COMMERCIAL PIGGERIES

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Introduction

In recent years, numerous applications in the framework of precision livestock farming for animal production have been reported. For example; Van Hirtum et al. (2001) describe a cough as a natural acoustic indicator of animal welfare. Other approaches examine the relationship between vocalisation (Van Hirtum and Berckmans, 2004), drinking behaviour (Madsen and Kristensen, 2005) or temperature (Geers et al., 1997) and animal welfare. It has been shown that pig vocalisation is directly related to pain and a classification of such sounds has been attempted by Marx et al. (2003). More recent research has focused on sound analysis from commercial piggeries in both their characteristics (Ferrari et al., 2008a; Ferrari et al., 2008b) and in creating automatic classifiers (Exadaktylos et al., 2008a; Silva et al., 2009) while work has also been performed in the localisation of the pig sounds (Silva et al., 2008) and in combining the localisation and classification algorithms (Exadaktylos et al., 2008b).

Objective

This paper provides an overview of the work done in sound analysis for health monitoring in piggeries.

Results and Discussion

In a first study, with experiments under laboratory conditions, algorithms have been developed to detect cough sounds from all other sounds. A sound-database of 5319 individual sounds including 2034 coughs was collected on 6 healthy animals containing both animal vocalisations and background-noises. This resulted in a positive cough-recognition of 92% (Van Hirtum et al., 2001). In the another study, the classification resulted in correct classification of 85.5% for the cough sounds and 86.6% correct classification of the other sounds (Guarino et al., 2008). In the second step, existing cough identification methods was extended and proposed a real-time method for identifying sick pig cough sounds. In total, 11 experiments were conducted to 3 male and 3 female healthy Belgian Landrace piglets of 9–12 weeks of age and 20–40 kg of weight. The generated data set includes individual sounds of 231 chemically induced coughs, 291 sick coughs, 149 other sounds. Preliminary results for the evaluation of the algorithm are based on individual sounds of healthy and sick animals acquired in laboratory conditions. An 85% overall correct classification ratio is achieved with 82% of the sick cough sounds being correctly identified (Exadaktylos et al., 2008a).In the last step, the position algorithm was applied on the localization of coughing pigs in a stable in field condition. Using this method it was possible to localize cough attacks of pigs. During a 3h recording trial, soundwas recorded using seven microphones in which 179 coughs were collected, originating from 19 cough attacks. After mapping the locations in the stable, some hazard zones could be identified. Although the algorithm is able to show the locations of the coughs (cough attacks), this does not mean the coughs originate from different pigs. It might be that different coughs, which were recorded in a pen, possible from a single pig. All the configurations showed good position estimation, with mean error between 1.5 and 0m, and a maximum standard deviation on the error of 0.4 m (Silva et al., 2009).The ability of detecting and localizing cough sounds and the increasing importance of animal welfare and monitoring indicate that in the future such systems will become available. Not only will the veterinarians have a quantitative indication about condition of the pigs, but also the farmer would get continuous feedback on the pigs' condition by automatic on-line monitoring.

Conclusion

This methodology can be used for visualizing the spread of respiratory diseases and contribute to the reduction of the use of antibiotics by means of selective and early treatment of single pens instead of the whole compartment.

MODELLING THE INFLUENCE OF HUSBANDRY AND CONTROL MEASURES ON PORCINE CIRCOVIRUS TYPE 2 (PCV-2) DYNAMICS WITHIN A FARROW-TO-FINISH PIG FARM

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Introduction

Even if PCV-2 has been demonstrated as the aetiological agent of Post-weaning Multisystemic Wasting Syndrome (PMWS), additional factors seem to be necessary for clinical signs to develop. Epidemiological studies of risk factors of PMWS clearly evidenced the dynamics of PCV-2 infection in growing pigs as a pivotal event: the earlier the infection, the higher the risk [1]. Deviations in management procedures in the early life of growing pigs such as increased cross-fostering rate, mingling practices in nursery facilities or rearing pigs in large pens after weaning were identified as clear risk factors for PMWS and suggested that these conditions favoured pathogen transmission between pigs. PCV-2 commercial vaccines (sow- or piglet-targeted) are now available throughout the world and have shown their efficacy in the control of PCV-2 related disorders. The aim of this work was therefore to study the influence of several husbandry practices and vaccination policies on PCV-2 course of infection. A stochastic individual-based model has been developed to reproduce rearing conditions within a farrow-to-finish pig farm and coupled with an epidemiological model of PCV-2 infection.

Material & Methods

A stochastic individual-based model was developed to describe population dynamics within a farrow-to-finish pig farm. This population model was built using a discrete-time simulation approach [2]. The epidemiological model is based on a classical SEIR model with 5 supplementary states representing the effect of passive immunity intake on the infectious process and the possibility for the piglets to be infected at birth. The most important parameters were derived from three experimental studies which led to the estimation of 6 transmission rates. The age at infection was studied by recording from simulations (n=1800 simulated batches per strategy) the ages at which piglets got the infection. The age-to-infection events were subjected to survival analysis. Strategies were tested by comparing survival distributions between strata and modelling the time-to-event using a Cox proportional hazard model (Proc PHREG, SAS 9.1).

Results

The most permissive strategy (random cross-fostering and mixing within large pens in the nursery) was taken as the reference for each comparison. The infectious agent was found to spread more slowly under all the other tested strategies, the hazard ratios ranging from 1, for the reference strategy, to 0.52 [0.46; 0.59] for the best one (no cross-fostering and grouping piglets by litters in small pens in nursery rooms). However, the risk of early infections was significantly decreased (HR=0.89 [0.80; 0.92]) by modifying just one parameter, such as the size of the pens in the nursery rooms. Vaccination was shown to significantly reduce the risk of early infections in the reference strategy setting. Sow-targeted vaccine delayed the infectious process until the loss of maternal antibodies at the end of the nursery phase, leading to a hazard ratio of 0.49 [0.40; 0.60]. Piglet-targeted vaccine reduced the force of infection and decreased the final number of infected animals (hazard ratio: 0.44 [0.37; 0.54]). However, the restrictive management strategy significantly improved the effects of both vaccination schemes providing hazard ratios of 0.35 and 0.24 for sow- and piglet-targeted vaccines respectively.

Discussion & Conclusions

Taking the most permissive situation as reference, it was found that the infectious process was delayed and the number of early infections reduced when the cross-fostering rate was reduced and the pigs were grouped by litters in the nursery rooms. Our simulation results also showed a huge impact of both vaccination schemes on PCV-2 course of infection with a dramatic decrease of the number of infections in case of piglet vaccination, leading to a potential pool of naïve animals as regards PCV-2.

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PORCINE CIRCOVIRUS TYPE 2 (PCV2) PREVALENCE IN ABORTIONS IN FRANCE

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Introduction

The role of porcine circovirus type 2 (PCV2) in reproductive disorders has been studied (1). The present paper reviews the results of the lab diagnosis collected through a Merial protocol "*pack avortons*" from June 2008 to June 2009.

Material and Methods

Aborted foetuses or weak piglets (AF-WP) include stillborns and weak piglets which breathed and/or suckled some colostrum before death.
Macroscopic aspect was detailed for 271 AF-WP. Due to poor conservation, 25 AF-WP were not submitted for any analyses.
PCV2, PPV and PRRSV diagnostics were performed on 296 AF-WP from 93 sows in 30 farrow-to-wean and farrow-to-finish non-PCV2-vaccinated herds.
Lab analyses for PRRSV and/or PPV were performed in 15 herds out of 30 on lung pools. In each herd, 1 pool (=3 AF-WP) for each test: all pools were PCR-negative.
Diagnostics for PCV2 were completed in 266 AF-WP as follows:
- by PCR on liver and/or heart for all pooled AF-WP (101 pools: 1 pool=max 3 AF-WP from the same dam)
- in frozen AF-WP which did not drink or breathe totally: anti-PCV2 antibody test performed on 135 AF-WP using thoracic and/or abdominal liquid (SERELISA® PCV2 Ab, Synbiotics, Lyon, France)
- by immunohistochemistry (IHC): 36 IHC were performed from individual AF-WP samples: 11 on livers and 25 on hearts.

A farm was considered as facing PCV2 reproduction disorders when at least 1 AF-WP was PCV2 positive using at least 1 method.

Results

Table 1: PCV2 prevalence in herds

	No/Total	Percentage (%)
PCV2-positive farms	12/30	40
PCV2-positive AF-WP	66/266	24.8

In the 66 PCV2-positive/266 AF-WP (24.8%) PCV2 was found most frequently in hearts, except in stillborn AF-WP in which anti-PCV2 antibodies were found. Anti-PCV2 antibodies were found in all AF-WP types except in mummified AF-WP and in AF-WP with lysed/discoloured liver.

Table 2: Distribution of PCV2-positive AF-WP according to organ and analysis techniques

Clinical aspect of positive AF-WP(No)	%age + liver (PCR &/or IHC)	%age +heart (PCR &/or IHC)	Abdom. &/or thor. liquid (ELISA)
No lesion (31)	34.6	51.6	69.6
Lysed (11)	0	85.7	50
Mummified (8)	100	75	0
Stillborn (8)	0	0	100
Lysed/discolored liver (3)	0	100	0
Breathed/no drink (5)	66.7	20	75
Total positive AF-WP (66)	29.1	51.6	61.5

Discussion

The *"pack avortons"* was put in place to systematically confirm or infirm the involvement of the major reproductive pathogens, i.e. PPV, PRRSV and PCV2 in case they could be suspected. All analysis methods taken together, these results report a high PCV2 prevalence. Indeed PCV2 was evidenced in AF-WP in 40% (often several aborted litters per case) of herds where reproduction disorders were investigated.
In addition, these results enabled to define a diagnosis strategy: mummified foetuses are more likely to be found PCR positive (mumified piglets are often dry so no liquid can be harvested from them to perform ELISA) especially when they are not lysed whereas stillborn foetuses are more often ELISA-positive (i.e. anti-PCV2 antibodies detection). It is well-known that foetuses become immunocompetent from the last third of the gestation, i.e. about 70 days onwards. So age (>70 days of age, i.e. about 17 cm long) and denaturation status of the foetuses could be used as choice criteria for the diagnosis method.

Conclusion

PCV2 appears to be involved in a large number of reproductive disorder cases and has to be included in the differential diagnosis.

Acknowledgements: the authors wish to thank the LDA22 laboratory.

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DESIGN, REPORTING AND CRITICAL INTERPRETATION OF CLINICAL TRIALS

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Introduction

"Clinical trials" are studies conducted to provide evidence for demonstrating and testing a causal relationship between an intervention (often a treatment) and an outcome. Clinical trials follow specified protocols including specific design elements such as blinding and randomization (see below). Out of all non-experimental study designs, they are the most controlled and therefore least affected by bias.

Clinical trials are conducted using patients that are recruited through hospitals and practices. Practitioners have access to such "real" cases and are therefore well positioned to conduct or contribute to clinical trials. Results from clinical trials contribute substantially to the development of novel substances and treatment regimes. It is therefore desirable that practitioners get involved.

When conducting clinical trial, ethical aspects of conducting research on patients have to be considered. Although this is not the focus of this paper, it should be highlighted that there are legal obligations and ethical principles that need to be adhered to. Most importantly, consent has to be obtained from the animal owner. This is typically achieved by providing the owner with a printed information sheet highlighting the elements and risks of the trial. The owner needs to formally give consent by signing this document. In large clinical trials, an end point needs to be defined and monitored to avoid continuing the trial when the advantage of either of the treatments is already established.

Design

Clinical trials are conducted under practice conditions using naturally occurring cases. The objective is to include cases that are representative of those for which inference about the treatment should be drawn. Nevertheless, **selection bias** can be an issue, i.e. a systematic selection of certain types of cases (for example, severe cases) which are not representative of all relevant cases. This is typically addressed by defining **inclusion and exclusion criteria** as well as detailed **case definitions** reflecting the range of severity relevant for the objective of the study. Also, the epidemiological unit needs to be defined (individual animal, batch, farm).

A special challenge is the risk of **confounding**, i.e. the association of the outcome with a factor that is not controlled in the selection process, for example, breed or environment. In clinical trials, **randomization** is the technical solution to prevent confounding. The objective of randomization is for all units in the trial (animals, farms, groups) to have an equal probability of being in either of the treatment groups. If randomization is correctly conducted, treatment and comparison group are comparable with respect to all characteristics even those that were not identified as a confounder. Clinical trials should have at least one **control group**. Without a control group, the effect of the intervention cannot be formally assessed.

While clinical trials are typically conducted prospectively, retrospective designs are sometimes described. The key problem with these is the lack of randomization. Results from non-randomised studies should be seen as cohort studies and their results interpreted with care as causal inference is normally limited.

The treatment group will receive the intervention of interest, and the control group will either receive a placebo or – most frequently – the treatment currently considered to be the best option. The target number of individuals/units in each group needs to be established using sample size calculation equations. The required sample size will depend on the expected difference in effect between treatment and control (larger difference – smaller sample), the level of confidence in the results (higher confidence – larger sample size) and the variability of the outcome in each group (higher variability – larger sample size). For more details on sample size calculations, see Elwood (3).

In order to avoid bias in outcome assessment and general treatment of patients, their group membership (treatment vs. control) is typically unknown to the attending personnel. This design feature is called **blinding** (or masking). If only the subject (in veterinary medicine, the owner) is "blind", this is called a **single-blind** study. If also the attending medical professionals (i.e. the observers) are "blind", this is a **double-blind** study. The latter is considered the standard. Blindness has to be maintained throughout the study. Sometimes, blindness cannot be achievable due to technical reasons,

for example, when one treatment requires surgery and the other does not.

The **outcome** of a clinical trial needs to be defined in detail including the description of diagnostic approaches to be used and time windows (follow-up time), if relevant. The outcome can be qualitative (recovered vs. not recovered with recovery being specifically defined) or quantitative, e.g. increase in feed conversion. Mortality (or survival) can also be a study outcome.

For quality assurance purposes, all aspects of the design and conduct of a clinical trial should be specified in the study protocol. The protocol is required in many countries as part of the ethics approval process. Management of data and assurance of data confidentiality are other important aspects of good research practice.

Reporting

Reporting of clinical trial results should follow the CONSORT statement (2). CONSORT stands for Consolidated Standards of Reporting Trials and aims at improving the quality of reporting. Reports should describe all elements of the trial and provide at least the information indicated in **Figure 1**.

As with results from other studies, the design of clinical trials needs to be reported such that the reader is able to fully reproduce the study. The "Material & Methods" section therefore needs to be sufficiently detailed. Again, more details are provided by CONSORT. Most medical and many veterinary journals apply the CONSORT criteria to manuscripts submitted for publication.

Depending on the outcome of the trial (qualitative or quantitative), the appropriate analytical methods will have to be chosen. If in doubt, consultation with a statistician is recommended. For binary outcomes (recovered / not-recovered, survived / not-survived, diseased / not-diseased), 2x2-tables are a good way of presenting the data (**Table 1**) and results can be presented as relative risk (or risk ratio). The relative risk (RR) is calculated as follows:

$$RR = \frac{A/A+B}{C/C+D}$$

Confidence intervals of RR must always be calculated and reported. In case of an effect of the treatment or intervention, the RR is expected to be >1 and the confidence interval should not include 1. Attributable risk (risk difference) and attributable proportion may also be useful to report (3).

Table 1. 2x2-table for presenting results of a clinical trial with recovery as an outcome

	Recovered	Not-recovered	
Treated	A	B	A+B
Control	C	D	C+D
	A+C	B+D	TOTAL

If the outcome is quantitative, the difference in the mean (or median, depending on the distribution of the data) between the groups must be calculated. There are free tools available on the internet for easy conduct of such analyses, e.g. WinEpiscope (4).

Critical interpretation

While only a few clinicians may become involved in the design, conduct and analysis of clinical trials, all will be confronted with results from such studies. This may either be in the context of continuing professional development when reading a journal article or in a discussion with a representative of a drug company who may use clinical trial results to encourage a sale. The ability of critical interpretation of such results may therefore be directly linked with clinical decisions and ultimately economic benefits (or losses).

Two key criteria when considering results of a clinical trial are internal and external validity. **Internal validity** is achieved, when differences between the groups in the study reflect true differences. Internal validity depends on the study design, randomisation and diagnostic procedures. **External validity** is a pre-requisite for extrapolating results from a study to the target population. It primarily depends on the representativeness of the cases included.

Finally, results of a clinical trial should be interpreted in the context of clinical relevance and the economic considerations. Such assessments can include results across several trials leading to so-called meta-analyses and form the basis for evidence-based medicine (1).

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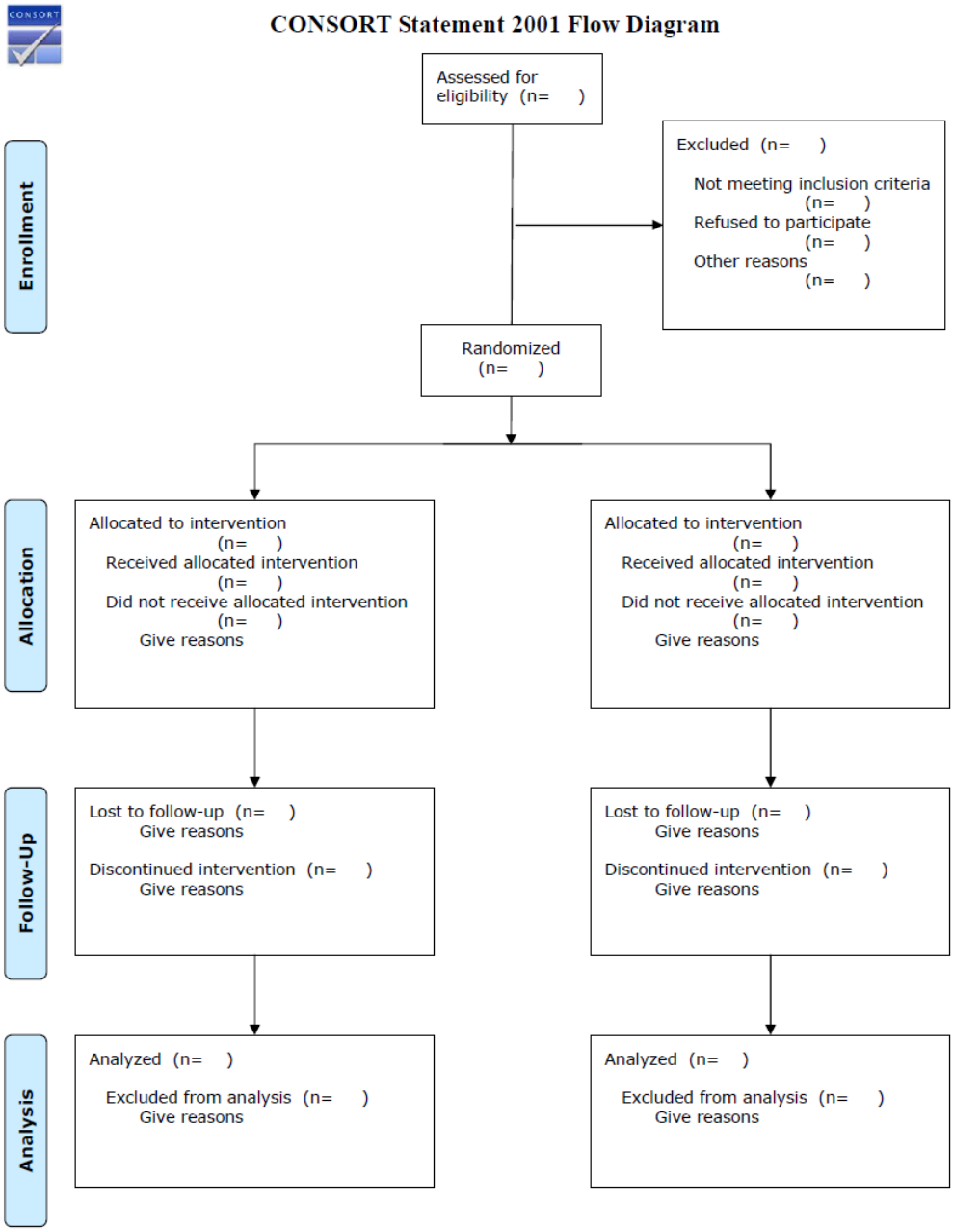

Figure 1: CONSORT recommended flow chart for describing a randomized trial (www.consort-statement.org)

COMPARISON OF BACTERIOLOGICAL FINDINGS IN PIG HERDS WITH HIGH OR LOW SALMONELLA ANTIBODY PREVALENCES

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Introduction

In 2007, Germany established a national serological monitoring program for *Salmonella* in accord with the EU zoonosis regulation from 2003. All herds with finisher pigs must take part. After the first sixty samples have been taken and analyzed, the herd is then categorized as having either high (category III), intermediate (category II) or low (category I) risk of introducing *Salmonella* into the food chain. The farmers of category III herds are compelled to find the source of the *Salmonella* problem as well as residual contaminations and to implement measures against the bacteria.

Basing sampling on known areas of contamination (1, 2), one goal of this study was to see if the environment of herds in category III is indeed more contaminated with *Salmonella* than that of herds in category I. Because herd management and especially correct cleaning and disinfecting measures are seen as critical part of combat strategy against *Salmonella* (3, 4), when ever possible samples were also taken after cleaning and disinfection had taken place.

Material & Methods

In category I 67 herds were examined; in category III 103 herds. Samples (n=3071) were collected by swabbing the area in question with a sterile, buffered peptone water (BPW)-enriched tissue. The samples were then examined via RT-PCR, after an 18 hour enrichment phase in BPW at 37°C at the Field Station for Epidemiology.

Results

The following tables show merely an excerpt of the obtained results.

Table 1 total samples

Table 2 samples after cleaning and disinfection

Table 1

Sample	Sum	Positive	Cat. III	Cat. I
Faeces from pen floors	451	20,8%	25,0%	9,8%
Hallways outside pens	260	28,5%	34,7%	15,5%
Feeders and Troughs	264	24,6%	29,3%	17,8%
Driving boards	239	20,5%	26,0%	11,2%
Pig toys	234	19,7%	23,7%	15,5%
Pen walls	211	23,7%	27,3%	18,9%

Table 2

Sample	Sum	Positive	Cat. III	Cat. I
Hallways outside pens	20	30,0%	30,8%	28,6%
Feeders and Troughs	43	18,6%	29,2%	5,3%
Driving boards	12	16,7%	22,2%	0,0%
Pig toys	27	22,2%	23,1%	21,4%
Pen walls	28	17,9%	18,8%	16,7%

Discussion & Conclusions

The bacteriological findings show that the category III herds are indeed in general more contaminated with *Salmonella* than the herds in category I. This holds true as well for the samples after cleaning and disinfection, even if the total number of samples here is not great. These results lead to two interesting questions:

1. Do category III herds not have as a good hygiene strategy as category I herds?
2. Or is it simply a problem of the correct and consistent execution of measures already in place?

The fact that herds in category I are not *Salmonella* free shows that no farmer can be negligent in his biosecurity measures merely on the basis of “good” serogical results, but that the successful battle of this zoonosis (and others) rests on consistent vigilance.

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EFFECT OF ORGANIC SELENIUM IN THE DIET ON SPERM QUALITY OF BOARS

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Introduction

Selenium (Se) is known to be a component of glutathione peroxidase (GPx) which acts as an antioxidant. In sows, Se deficiency leads to reproductive failure [1]. On growing boars, supplementing the diet with Se resulted on a beter semen quality as well as an increased GPx activity in sperm [2]. Recently, Jacyno *et al.* 2002 [3] compared the effect of different sources of Se (organic as selenomethionine vs inorganic as selenite) on sperm quality of growing boars (70 -180 days). The latter study demonstrated for the organic Se group higher total sperm/ejaculate, less morphology abnormalities but no differences in motility. However, for the latter study, subjective methods to assess motility where used.

The aim of this study was to assess the possible beneficial effects of a diet supplemented with organic Se in the feed of good performing mature boars on semen production and quality. Different semen parameters such as volume of the ejaculate, the concentration, motility and morphology of the sperm and the resistance to oxidative stress were investigated.

Material & Methods

Sixty mature boars from a commercial artificial insemination (AI) centre were randomly allocated into group A (GA) and B (GB). GA continued with the previous feed [premix with inorganic Se (0.4 mg Na2SeO3/kg) and vit E (80 mg/kg)] whereas GB was switched to a commercial premix [organic Se (0.4 mg/kg, Se-yeast, Selplex®, Alltech) and vit E (80 mg/kg)]. The sperm was investigated during 4 months (D0, D30, D60, D90, D105 and D120). The following parameters were studied: sperm concentration (x10^6/ml; photometer IMV Accucell); motility [Computer Assisted Semen Analysis-Hamiltone Thorne (CASA-HTR)]; morphology (eosin-nigrosin staining), oxidative stress [production of malonaldehyde (µg MDA/l) with thiobarbituric acid reagent species (TBARS)] and Se concentration in sperm cells and blood plasma. Repeated measures ANOVA from D60-120 (spermatogensis of aprox. 2 months) or ANOVA at D120 (Se concentrations) were used for statistical analysis

Results

Differences (P<0.05) were observed in: sperm concentration (GA: 434.6; GB: 514.1; Fig 1); oxidative stress (GA: 293.2; GB: 351.5; Fig 2); CASA-HTR straight-line velocity [µm/s (GA: 48.3, GB: 45.1], straightness [% (GA: 65.6, GB: 62.2)] and linearity [% (GA: 32.2, GB: 29.3]. No differences (P>0.05) were shown for the other parameters

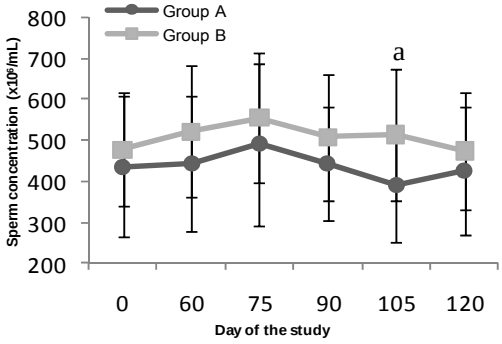

Fig. 1: Average (±SD) sperm concentration (x10^6/ml, IMV Accucel®) of ejaculated boar sperm in Group A (n=28) and Group B (n=28) at different time points. Group A received a diet based on a premix with inorganic Se (0.4 mg Na_2SeO_3/kg) whereas Group B received a diet based on a premix with organic Se (0.4 mg Se-yeast/kg).There was a significant overall difference in the repeated measures analysis between the 2 groups from Day 60 to Day 120 (P<0.05). [a]Timepoints with significant differences between the two groups (P<0.05)

Discussion & Conclusions

It appeared that changing from inorganic Se (GA) to organic Se (GB) in the diet only induced minor changes in a limited number of the measured parameters. Boars from Group B had a higher sperm concentration but some motility parameters and the resistance to oxidative stress were lower. Other parameters were not significantly influenced.

Higher TBARS could be due to lower GPx activity in the organic group. Selenium incorporates into GPx as selenocysteine whereas selenomethionine is the form in which Se is presented in the organic Se. Selenomethionine enters the body as a pool of methionine and competes with methionine for incorporation in non-Se-requiring proteins. Therefore Se is in this way less available for synthesis of GPx [4].

Why sperm concentration was higher in the organic group is difficult to explain. The accuracy of methods to analyse sperm

concentration is widely discussed [5]. However a proven method and same conditions were applied for both groups thus biases are unlikely.

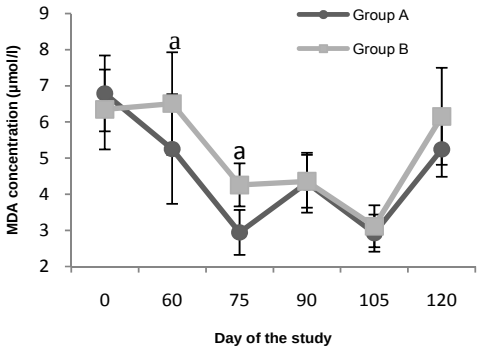

Fig. 2. Average (±SD) malondialdehyde (MDA) concentration (lmol ⁄ l) in sperm from extended sperm samples after lipid peroxidation induction with ferrous sulphate in Group A (inorganic Se; n = 8) and Group B(organic Se; n = 10). There was a significant overall difference in the repeated measures analysis between the 2 groups from Day 60 to Day 120 ($P<0.05$). [a]Timepoints with significant differences between the two groups ($P<0.05$)

Regarding the differences on CASA parameters, a recent study showed negative association between TBARS and motility [6]. However, such association was not confirmed in this study.

In conclusion, changing from inorganic to organic Se in the diet of good performing boars resulted in minor changes on sperm. Sperm from boars fed inorganic Se showed a more straightforward movement and higher resistance to lipid peroxidation whereas the sperm from de organic Se group showed higher sperm concentration.

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RECENT ANTIMICROBIAL SENSITIVITY DATA OF *BRACHYSPIRA HYODYSENTERIAE* IN BELGIUM: AN ANALYSIS OF EVOLUTION

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Introduction

Evolution of antimicrobial resistance (AMR) of *Brachyspira hyodysenteriae*, especially to pleuromutilins, should be monitored regularly to update the actual treatment possibilities in case of clinical disease or eradication strategies. A study, using strains isolated in 2003, on the link between AMR and clinical effect of treatment to *B. hyodysenteriae* infections revealed that only 13% of the strains tested were susceptible to lincomycin and just 4% to tylosin (1). Since 2003, no recent data are available on *B. hyodysenteriae* AMR in Belgium. Therefore, we studied (project 'Veepeiler-varken' funded by the Sanitary Fund) the susceptibility of recently isolated strains of *B. hyodysenteriae* to the pleuromutilins tiamulin and valnemulin (3). This study showed a number of resistant *B. hyodysenteriae* strains to tiamulin and in a lesser extent to valnemulin. Since 2006, recent data are yearly available (3) on *B. hyodysenteriae* AMR to tiamulin and valnemulin in Belgium.

Materials and Methods

Strains of *B. hyodysenteriae* were isolated from routine diagnostic samples during 2006-2008-2009 and immediately after isolation, AMR testing was performed. Besides the field isolates, a reference *Brachyspira* strain was also tested (2). Susceptibility testing was performed as previously described (1, 2). The minimal inhibitory concentration (MIC) was recorded as the lowest concentration at which no distinct hemolysis was seen in the spot in comparison with the hemolytic effect on the antibiotic-free control plates.

Results

Results of the field isolates of the different *B. hyodysenteriae* strains (n = 40 strains per year) are given in Figure 1 for tiamulin and in figure 2 for valnemulin. Minimal inhibitory concentrations for 50% (MIC_{50}) and 90% (MIC_{90}) of the strains tested are also given in Table 1. From Figure 1, it is clear that in recent years (2008-2009) an increasing percentage of *B. hyodysenteriae* strains have an MIC-value above 4 µg/ml. For valnemulin, the maximal MIC-value in 2006 was just 1 µg/ml. A rather rapid evolution could be observed in recent years (2008-2009) with an increasing percentage of strains having an MIC-value of > 1 µg/ml.

Table 1. Minimal inhibitory concentrations of tiamulin and valnemulin required to inhibit 50 and 90% (µg/ml) of *B. hyodysenteriae* strains isolated in Belgium in 2006-2008-2009

	Tiamulin		**Valnemulin**	
Year	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
2006	0.25	2	0.03	0.50
2008	0.50	8	0.12	8
2009	>8	>8	8	>8

Discussion

In comparison with previous studies (1), MIC_{50} and MIC_{90} were significantly increased for *B. hyodysenteriae*. Considering that isolates with MIC $\geq$ 1 µg/ml should be regarded as not responding to therapy *in vivo*, more than 50% of all isolates obtained in 2009 should be considered resistant to both tiamulin and valnemulin (1).

For valnemulin, the number of resistant strains (n = 27) significantly increased last year and became nearly as high as for tiamulin (n = 29). The fact that an increasing number of strains reaches MIC-values beyond the highest concentrations (MIC > 8 µg/ml) tested for both pleuromutilins is a serious concern for the Belgian pig industry. Especially in relation to eradication protocols, further research focussing at potential alternative control measures is necessary. Farms should be aware to further improve their external en internal biosecurity measures to prevent introduction of new *B. hyodysenteriae* strains in the farm.

In conclusion, antimicrobial resistance to pleuromutilins has changed towards more resistance and higher MIC_{50} and MIC_{90} values as compared to previous data, especially for tiamulin which is considered first choice antibiotic in the treatment of swine dysentery in Belgium.

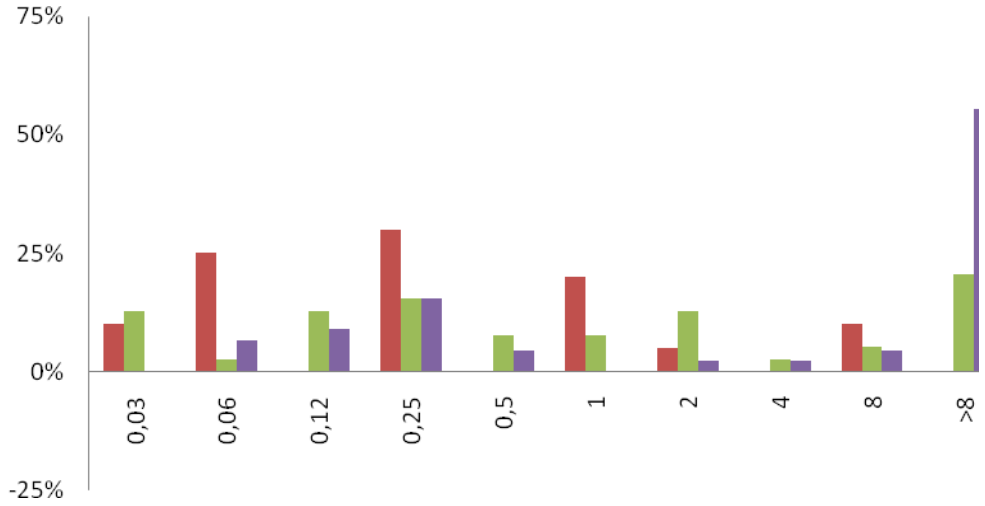

Figure 1. Percentage of strains with their respective minimal inhibitory concentrations of tiamulin for *B. hyodysenteriae* strains isolated in Belgium in 2006 (red) -2008 (green) -2009 (purple)

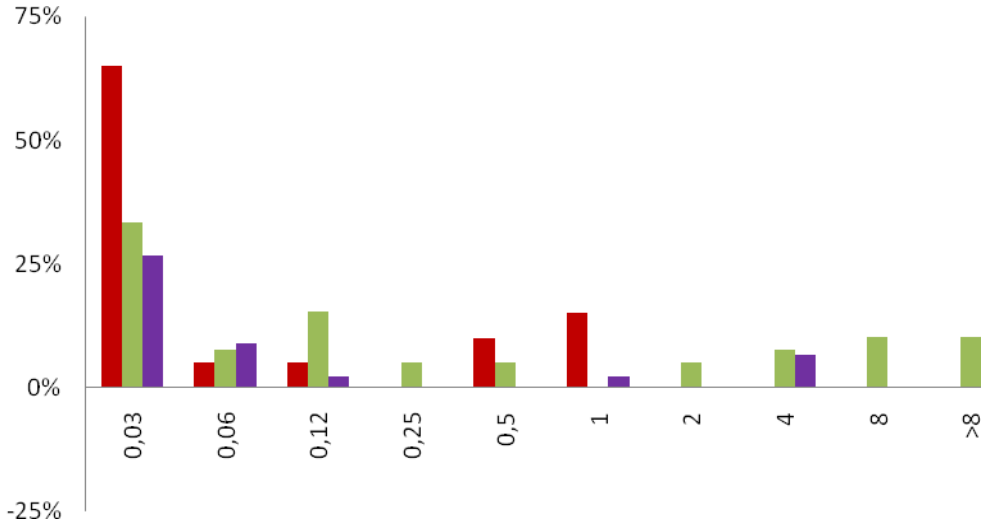

Figure 2. Percentage of strains with their respective minimal inhibitory concentrations of valnemulin for *B. hyodysenteriae* strains isolated in Belgium in 2006 (red) -2008 (green) -2009 (purple)

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QUANTITATIVE ASSESSMENT OF *LAWSONIA INTRACELLULARIS* BY PCR IN OUTBREAKS OF ACUTE DIARRHEA IN WEANERS

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Introduction

Lawsonia intracellularis (LI) is the causative agent of proliferative enteropathy (PE). PE was first described in pigs in 1931 and is now recognised as one of the most economically important diseases in the swine industry worldwide.

LI cannot be cultured by routine methods and the gold standard for diagnosing PE is demonstration of proliferative intestinal lesions associated with intracellular curved bacteria in histological sections. In the last 15 years, progress has been made in the development of PCR tests for detection of LI in feces. These tests are now commonly used for routine diagnosis of PE in diagnostic laboratories.

Field and experimental studies have reported fecal PCR detection of LI in the absence of clinical signs and production losses. Further, simulated predictive values for 6 published PCR tests suggested that applying the fecal PCRs as a diagnostic test is more likely to overestimate than underestimate the number of pigs having histological lesions of PE under field conditions (1). These results imply that interpretation of the traditional PCR tests are difficult in terms of importance for the individual pig and at herd level. Recently development of quantitative real-time PCR (q-PCR) tests (2) for LI in feces have been reported. These tests allows quantitative assessment of excretion in the individual pig which may help in diagnosis of PE. In order to interpret results of q-PCR for LI knowledge of excretion levels in relation to clinical status is needed.

The objective of the current study was to investigate the quantitative fecal excretion of LI during outbreaks of acute diarrhea in weaners.

Material & Methods

A case control study was designed. Herds were selected by multistage sampling. All herds serviced by six specialized swine veterinarians from the same vet practice at Zealand and fulfilling the inclusion criteria were selected. The criteria were recurring therapeutic use of in-feed or in-water medication for diarrhea at room level in pigs between 10 and 70 days post weaning. Only herds representing intensive production systems were selected. One outbreak of acute diarrhea was investigated in each herd. All herds were visited the day following notification from the farmer/veterinarian of an acute treatment requiring outbreak of diarrhea and the farmer was not allowed to medicate before the pigs were examined. If the pigs had received antibiotic batch medication within the last 7 days of the examination day, the outbreak was excluded from the study. A sample of 80 pigs in each herd was selected by systematic random sampling among all pigs in the nursery room where the outbreak occurred. The selected pigs were subjected to a clinical examination and fecal samples were collected. Among the examined pigs a simple random sample of 20 pigs with diarrhea and 20 pigs without diarrhea was selected for q-PCR testing and fecal dry matter (DM%) determination by microwaving. DM% ≤ 18.8 was considered as diarrhea (3) and was used (if necessary) to reclassify the pigs as diarrheic (cases) or non-diarrheic (controls) in the statistical analysis. The DM% was further applied to estimate the true diarrhea prevalence in the outbreaks by calculation of the observer's diagnostic sensitivity (Se) and specificity (Sp) for detection of diarrhea. Statistical analysis included Chi-sq tests, linear regression and linear mixed models. All analysis was performed in STATA IC, version 11.

Results

A total of 14 outbreaks of treatment requiring diarrhea were investigated in 2009. Clinical examination was performed on 1106 pigs. The observer's diagnostic sensitivity and specificity for detection of diarrhea were 0.89 and 0.92 respectively. The true prevalence of diarrhea was 38.3% (range of within outbreak prevalence: 20.6-72.9%). The examined pigs were on average 31 days post weaning (range: 15 - 63 days). A total of 541 pigs were examined by q-PCR. LI was detected in 28.3% of the pigs. The prevalence of LI was significantly different between outbreaks ($p<0.001$). LI was not detected in 6 outbreaks. The outbreaks with detection of LI (n=8), fell in two distinct groups (low/high prevalence) in relation to prevalence of LI in pigs with diarrhea, table 1. The within outbreak prevalence of LI in pigs with diarrhea was significantly different between outbreaks

Table 1. Prevalence (%) of LI detected by q-PCR in outbreaks of acute treatment requiring diarrhea. Range of within outbreak prevalence is displayed in ( )

	Diarrheic pigs (DM%≤ 18.8)	Non-diarrheic pigs (DM%>18.8)
Low prevalence outbreaks (n=3)	11.9 (9.1-15.8)	11.5 (4.6-23.8)
High prevalence outbreaks (n=5)	79.6* (68.8-90.0)	65.0* (34.8-78.3)

*Chi-sq test (p=0.024)

classified as low and high. The LI prevalence in pigs with diarrhea was not significantly different between outbreaks with the same classification. The q-PCR positive pigs were on average 40 days post weaning (range: 20 – 63 days) compared to 27 days for the negative pigs (range: 12 - 63). The pigs in the high prevalence outbreaks were on average 43 days post weaning (range: 35-63) compared to 24 days (range: 12-48) for the pigs in the other outbreaks. In the high prevalence outbreaks pigs with DM%≤13.1 had a higher prevalence of LI (91.2%) than pigs with DM%>13.1 (67.9%) ($p<0.05$). For pigs with DM%>13.1 no association between different DM% and detection of LI was observed.

The excretion data for LI was log10 transformed to obtain normal distribution. All excretion data are displayed in Log10 LI cells/gram feces. The mean excretion in the low prevalence outbreaks (mean=4.21; standard deviation (sd)=1.20) was significant different from the mean in the high prevalence outbreaks (mean=5.18; sd=1.29) ($p=0.008$). In the investigated high prevalence outbreaks the mean excretion was significantly different between outbreaks ($p<0.001$) and decreasing DM% was associated with an increasing excretion of LI ($p=0.002$), figure 1. After taking the variation due to DM% into account the between outbreak variation accounted for 21.3% of the total variation in the quantitative excretion. Variation between pens within high prevalence outbreaks accounted for 6.47% of total variation in excretion.

Discussion & Conclusions

In Denmark it has been a common belief that LI is the primary pathogen involved in acute outbreaks of diarrhea in weaners. In the current study LI was detected in high prevalence in less than half of the investigated outbreaks. The study only represents a small sample of Danish herds, but the results do imply that other etiologies may also be common causes of diarrhea outbreaks in weaners. In the outbreaks with a high prevalence of LI the pigs were in the second half of the nursery period. This is also in contrast to the common belief that LI is a frequent cause of diarrhea in the first half of the nursery period. The high prevalence of LI in pigs without diarrhea may imply that most pigs within a batch of pigs get infected in a short time during an outbreak of diarrhea. Further investigations are needed to describe the proportion of such pigs developing diarrhea during the progression of an outbreak. The association between q-PCR and DM% (diseases severity) should be further investigated in order to determine the effect on health and production of different q-PCR levels. The significant higher prevalence in pigs with DM%≤13.1 implies that samples for demonstration of LI should be obtained from pigs with watery diarrhea. The quantitative excretion of LI was quite different between outbreaks (herds). This has implication for establishment of a cut-off for q-PCR as a diagnostic test. The importance of the variation between outbreaks in terms of disease and productivity needs further evaluation. The low variation between pens implies that effect of pens does not need consideration in interpretation of q-PCR results.

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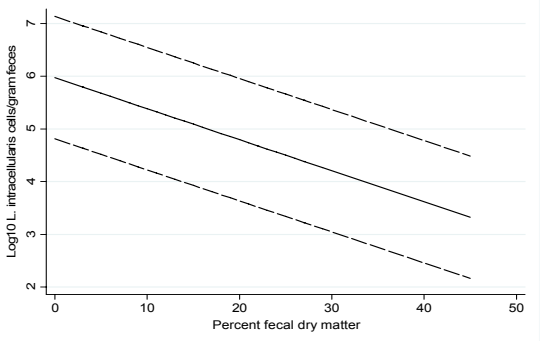
Figure 1. Linear relationship between DM% and mean excretion of LI (Log10 cells/gram feces) in an average high prevalence outbreak (solid line). The dashed lines represents the lower and upper limits for mean excretion in 95% of all high prevalence outbreaks.



Quality assurance in veterinary pig practice

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Introduction

Quality assurance (QA) may be defined as the systematic monitoring and evaluation of a process in order to ensure that standards of quality are being met. Quality refers to the expectations consumers/legislation may have regarding a certain product. This includes both the technical features of the product, the production process from which the product originates and the way they both are perceived.

European pig production is subjected to QA in several ways. EU legislation requires that the feed companies, the slaughterhouses, the food processing companies and the retailers establish advanced QA programmes mainly focusing on food-safety. The "missing link" in this food chain is the primary (pig) production, where QA systems so far are less strict and documentable than in the other parts of the food production chain. At present voluntary QA programmes are sufficient in many herds. Herds preparing home mixed feed based on "feed additives and pure substances" must, however, establish a more advanced QA system for this particular activity.

Veterinary pig practices may implement "Good Veterinary Practice" or other types of QA programmes. At present this is not widespread, probably due to the fact that there is no legal requirement, and that very few pig producers are demanding this kind of certification from practising vets. Following implementation of more advanced QA programmes in the primary production, the need for QA accreditation of veterinary practices will probably increase. This development is already taking place in veterinary poultry practice.

At present EU legislation does not require that pig producers involve veterinary practitioners in the establishment and inspection of QA programmes. Consultancy on implementation and inspection of QA programmes in pig herds is, however, a growing business.

Danish legislation will in the near future require that practicing vets performing herd health and production services based on contracts with pig producers must also advice on and control QA programmes. These so-called "own check programmes" will be focused on animal welfare. This means that QA consultancy will be an integrated part of veterinary services.

Types of quality programmes in pig production

Quality programmes may be based on either quality control or quality assurance approaches. Quality control emphasizes testing of finished products to uncover defects in order to stop the release of the product. Quality assurance attempts to improve and stabilize production, and associated processes, to minimize issues that lead to the defects. An example of a quality control approach is traditional meat-inspection without ante-mortem information from herds. Examples of quality assurance programmes include HACCP and ISO 9000 (se below). The trend is to move from quality control to quality assurance programmes in order to focus more on prevention of defects than detection of defects when it is too late. Quality control methods are, however, most often an integrated part of a given quality assurance programme.

Concepts for QA programmes include: Good manufacturing practice (GMP); International standardization organization (ISO); Hazard analysis critical control points (HACCP).

Noordhuizen et al. 2005 has compared different types of QA programmes in dairy herds (Table 1). They concluded that a HAACP-like programme should be preferred since it is highly farm specific, easy to link up with management, relatively low in cost and well fit for certification.

Table 1. *The most relevant characteristics of three quality control concepts for application at dairy farms (Noordhuizen et al. 2005)*

Characteristic	GMP	HACCP	ISO
Approach	Top	Bottom	Top
Orientation	Process	Process	System
Farm-specific	No	Yes	No
Simplicity level	Moderate	Yes	No
Self-management level	Moderate	High	Low
Corrective measures	No	Yes	Yes
Labour demanding	Low	Moderate	High
Expected costs	Low	Low	High
Easy to link to	Moderate	High	Low
Operational Management documentation	Low	Moderate	High
Easy to link to Food chain	No	Yes	Yes
Health demonstrable	No	Yes	Yes
Fit for certification	No	Yes	Yes

GMP

Good manufacturing practice (GMP) is part of a quality system covering the manufacture of products. GMP guidelines are a series of general principles that must be observed during manufacturing. When a company is setting up a quality program it is the company's responsibility to determine the most effective and efficient quality process. The GMP programmes in pig herds are based on so-called sector codes or “codes of practice“. Examples of such codes are the German QS (Qualität und Sicherheit) system, and the code of practice used in Denmark called “DANISH”. These systems are audited by independent third party auditors.

HACCP

Hazard Analysis Critical Control Point (HACCP) is a quality assurance principle that focuses on planned prevention of potential hazards in a production process. It was developed for use in food safety, originally by a company supplying food for astronauts to NASA. Mortimer and Wallace 1994 describe HACCP in brief in this way:

- Look at your process from start to finish
- Decide where hazards could occur
- Put in controls and monitor them
- Write it all down and keep records
- Ensure that it continues to work effectively

HACCP has been formally adopted by the EU to assure food safety in different kinds of industries. Von Borel et al. 2001 have described a system for on farm assessment of critical control points in pig herds based on HACCP principles. It may be claimed that HACCP is just what a really good farmer has been doing anyway.

The HACCP seven principles

Principle 1: Conduct a hazard analysis. Determine the food safety hazards and identify the preventive measures to be applied to control these hazards. A food safety hazard is any biological, chemical, or physical property that may cause a food to be unsafe for human consumption.

Principle 2: Identify critical control points. A Critical Control Point (CCP) is a point, step, or procedure in which control can be applied and, as a result, a hazard can be prevented, eliminated, or reduced to an acceptable level.

Principle 3: Establish critical limits for each critical control point. A critical limit is the maximum or minimum value to which a hazard must be controlled at a critical control point to prevent, eliminate, or reduce to an acceptable level.

Principle 4: Establish critical control point monitoring requirements. Monitoring activities are necessary to ensure that the process is under control at each critical control point. Each monitoring procedure and its frequency should be listed.

Principle 5: Establish corrective actions. These are actions to be taken when monitoring indicates a deviation from an established critical limit.

Principle 6: Establish record keeping procedures. The HACCP programme requires that certain documents, including hazard analysis and written HACCP plan, and records documenting the monitoring of critical control points, critical limits, verification activities, and the handling of processing deviations is maintained.

Principle 7: Establish procedures for ensuring the HACCP system is working as intended. Validation ensures that the producers do what they said they would do. This may be done by an own check programme or by external audit. Verification ensures the HACCP plan is adequate, that is, working as intended. Verification also includes the process of finding evidence for the accuracy of the HACCP system (e.g. scientific evidence for critical limits).

ISO 9000 system

The International standardization organization (ISO) defines standards for a great number of processes from different businesses. ISO 9000 is a set of standards for quality management systems. Some of the requirements in ISO 9001:2008 (which is one of the ISO 9000 standards) include:

- A set of procedures that cover all key processes in the business
- Monitoring processes to ensure they are effective
- Keeping adequate records
- Checking for defects, with appropriate and corrective action where necessary
- Regularly reviewing individual processes and the quality system itself for effectiveness and
- Facilitating continual improvement

As seen from the list the are many overlaps between HACCP and ISO standards, and in practice a HACCP approach may be included in an ISO programme.
A company or organization that has been independently audited and certified to be in conformance with ISO 9001 may publicly state that it is "ISO 9001 certified" or "ISO 9001 registered". Certification to an ISO 9001 standard does not guarantee any quality of end products and services; rather, it certifies that formalized business processes are being applied.

Own check programmes

The principle of an own check programmes is that the company is responsible for its own day to day control. This means that it must be able to document that the quality standards has been met. Own check programmes have been used extensively to control food safety in food producing companies. Such programmes may be based on a good manufacturing practice programme. A written own check programme is required for areas where there is a direct risk for the food safety. Own check programmes are checked by the authorities or other external auditors in order to make sure that they function as required.

Own check programme may also be based upon the principles of HACCP (Hazard Analysis Critical Control Points). This implies e.g. risk analysis, documentation of the monitoring and establishing corrective actions.

More recently these programmes have been implemented to control that animal welfare in pig herds meets certain quality standards.

Herd health and QA programmes

In order to establish a HACCP-like QA programme in a pig herd it is necessary to collect systematic data. These data may include production levels, disease occurrence, mortality figures, antibiotic consumption etc. Since the same data are necessary for herd health and production management the two systems behaves synergistically. A QA programme may work as an integrated part of a herd health programme.

In Denmark the pig producers may sign a voluntary veterinary advisory service contract with a practicing veterinarian, which gives the right to treat diagnosed diseases under certain conditions. If such a contract has been made,

the veterinarian must visit the farm not less than 12 times a year. The objectives of these contracts are to improve the standard of health of the herd, to minimize the risk of infectious diseases, and to optimize the use of antibiotics in order to prevent development of bacterial resistance. It has recently been decided that additional to these duties the practicing veterinarian must audit and advice on the farmers own check programmes on animal welfare.

EU Legislation with relation to QA

EU directives and regulations have a strong focus on QA programmes. Food safety, feed, animal welfare and control programmes are described in the following list of EU legislation:

Animal protection:
Council directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes.

Food:
Regulation (EC) No 178/2002 of the European parliament and of the council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

Feed hygiene:
Regulation (EC) No 183/2005 of the European parliament and of the council of 12 January 2005 laying down requirements for feed hygiene.

Food hygiene:
Regulation (EC) No 852/2004 of the European parliament and of the council of 29 April 2004 on the hygiene of foodstuffs.

Meat hygiene:
Regulation (EC) No 854/2004 of the European parliament and of the council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption.

Control of feed, food, animal health and welfare:
Regulation (EC) No 882/2004 of the European parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

Conclusion

Pig production is a part of the human food-chain from farm to fork. Public health including food-safety and animal welfare including animal health are main concerns for the consumers/legislators. QA programmes are well suited to document that certain food safety and animal welfare standards are met. EU legislation focuses on GMP, HACCP and own check programmes to be implemented. QA is also a management tool for pig producers that want to focus on prevention, risk reduction and a stable production.

So far, only few veterinary practices run a formalized programme to document the quality of their services. The need for this will probably increase along with further implementation of QA in the primary production.

It is important to realize that QA programmes do not, necessarily increase the quality of a product, but rather makes certain, that the quality is certified and uniform. In order to increase the quality of veterinary services to pig producers other activities such as post graduate education may be of great importance.

For the veterinary practitioner QA consultancy and audit of QA programmes based on HACCP-like and own check programmes will probably become an important service to pig producers in the future.

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A VALID DIAGNOSIS REQUIRES GOOD CLINICAL PRACTICE (GCP) AND GOOD LABORATORY PRACTICE (GLP)

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Introduction

A valid, or in other words, correct diagnosis in case of any disease in pig herds is a prerequisite for a long lasting success of therapies and/or prevention programs. Whether non-infectious or infectious diseases are suspected after assessment of clinical symptoms, there is usually a need for in-depth examination. This can be done by gross pathology on pigs combined with histological examination of tissue samples, and/or by submission of various samples to a laboratory. Only thereafter a presumptive diagnosis can be changed into a "valid" diagnosis. Herd attending veterinarians should take into account, that several factors might influence laboratory tests and in conclusion their own diagnosis. This has become more important since number and complexity of laboratory methods have tremendously increased during the last years.

In order to achieve a high standard in diagnosing diseases, both parties, practitioners and laboratories, should ideally comply with internationally recognized recommendations, they should implement a quality assurance system and they should maintain regular quality control of their own work.

Good Clinical Practice

Standardization and quality assurance should at least start, when further (extended) examination is planned by a practitioner. Questions regarding the aim of the examination and the way to achieve this aim should be answered in advance. Thereafter, the practitioners have to make decisions about the sample size, the sample site and the method to be used in the laboratory. Attention should also be paid to labelling, storage and shipment of samples. Unfortunately, there is no international recognized guideline available, really fitting in the requirements of daily work in a veterinary practice. Despite this lack of rules, practitioners may be advised to use guidelines followed in scientific studies in order to cope with the tasks of standardization and quality assurance:

Good Clinical Practice (GCP) is intended to be an international ethical and scientific quality standard for designing, conducting, monitoring, recording, auditing, analyzing and reporting clinical studies evaluating pharmaceutical products. Compliance with this standard provides public assurance about the integrity of the clinical study data, and that due regard has been given to animal welfare and protection of the personnel involved in the study, the environment and the human and animal food chains. Any work should comply with points specifying the foreseeable risks and inconveniences for the individual trial subject, the safety, and well-being of the trial subjects. Trials should be scientifically sound, and described in a clear, detailed protocol. Moreover, a trial should be conducted in compliance with the protocol and individuals involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s). All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification. Finally, systems with procedures that assure the quality of every aspect of the trial should be implemented.

The core of this guideline is to ensure a sound scientific work in the field of pharmaceutical research and drug licensing process. However, several points emphasizing the need of thorough standardization and documentation may also apply to daily work of veterinary practitioners!

In terms of quality management including quality assurance and quality control, veterinary practices should keep a handbook ready describing its own structure, responsibilities of staff and general processes. This is comparable to the "study protocol" mentioned in the GCP guideline. As far as possible, the handbook should also contain detailed descriptions of all working processes, data collection forms and checklists suitable for documentation in the daily work. Standard operation procedures describing herd examination in case of different disease complexes should be provided (respiratory disease, enteric disease, reproductive failure, etc.) to assist a standardized and thorough

herd examination and documentation even by "young" or less experienced veterinarians.
There is also a need to explain the management and conduction of in-depth examination:

- How many and which animals should be selected for necropsy, blood sampling, nasal swabbing, etc.?
- What is a must in terms of labelling, storage and shipment of samples?
- Which additional information has to be send with the samples to the laboratory?

This list contains only few, but very essential points and extension is highly recommended!

Good Laboratory Practice

The need of standardization, quality assurance and quality control is also a prerequisite for "valid" laboratory diagnostics. Any trust between practitioners and laboratories can only be based on a transparent work state of the art and good communications both ways. Laboratories themselves should be independent and disengaged of any third party i.e. pharmaceutical companies. In order to achieve these aims, laboratories may also be advised to use guidelines followed in scientific studies in order to cope with the tasks of standardization and quality assurance:
Good Laboratory Practice (GLP) is defined in the OECD Principles as "a quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported." The purpose of the Principles of Good Laboratory Practice is to promote the development of quality test data and provide a tool to ensure a sound approach to the management of laboratory studies, including conduct, reporting and archiving. The Principles may be considered as a set of standards for ensuring the quality, reliability and integrity of studies, the reporting of verifiable conclusions and the traceability of data. The Principles require institutions to assign roles and responsibilities to staff in order to ensure good operational management of each study and to focus on those aspects of study execution (planning, monitoring, recording, reporting, archiving) that are of special importance for the reconstruction of a whole study.
Besides the core elements of this guideline, (veterinary) diagnostics requires a thorough validation of methods for the examination of clinical samples. In some cases, i.e. notifiable diseases or pharmaceutical studies, this is also demanded by official institutions, such as local accreditation organizations, the European Agency for the Evaluation of Medicinal Products (EMEA) or the Office International des Épizooties (OIE). The procedures to fulfil their requirements are published in numerous guidelines. Compliance with these criteria might not be necessary if other than official reasons are the purpose of investigation. However, the use of a guideline for validation procedures, although optional, ensures that important physical characteristics of a new method get well assessed. Moreover, a transparent and structured validation reflects a proper quality assurance, which is more and more demanded in the scientific community, and, thus, is strongly recommended!

Discussion & Conclusion

A valid diagnosis in diseased animals should be based on good clinical and good laboratory practice. Although both parties, practitioners and laboratories, cannot fulfil all the requirements mentioned in the original GCP and GLP guidelines, which even do not match all circumstances in their daily work, a compromise between practicability and necessity is recommended:
Practitioners and laboratories can build up their own system of standardization, quality assurance and quality control. These management systems and their content have to be documented and should be, at least, conform to ISO/IEC 9001:2004, which is a internationally recognized guideline describing the requirements of Quality Management Systems. Furthermore, laboratories should work in compliance with ISO/IEC 17025:2005. This guideline specifies the general requirements for the competence to carry out tests and/or calibrations, including sampling. It covers testing and calibration performed using standard methods, non-standard methods, and laboratory-developed methods.
The conformity of all processes should be regularly certified by a neutral organization. This certification and the transparency of all activities will enhance the quality of conducted diagnoses and will emphasize the trust between all parties. Finally, diagnostics will become comparable within the EU and should be accepted across any borders.

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QA IN DUTCH PIG PRODUCTION

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Introduction

For more than 30 years the Dutch pig producers export pigs and pork to a lot of European countries, especially Germany, Italy and Spain. The Dutch pig producers are famous for the excellent knowledge of pig production, but guarantees on the health status of the pigs and the quality of pork are still needed. Therefore the Dutch Government and the Dutch Livestock Board have developed several systems. The aim of this paper is to give an overview of the surveillance systems on pig health and of the quality assurance system of pig production.

1. Surveillance on pig health (national level).

To gain more insight in the health status of the pigs on Dutch farms the Dutch Ministry of Agriculture and the Dutch Livestock Board (PVV) built a surveillance system for pig health in The Netherlands. The GD-Animal Health Service (GD-AHS) was asked to carry it out. The goals of this system are 1) watching trends and developments, 2) detecting outbreaks of notifiable diseases and 3) detecting unknown diseases/problems.

The surveillance system consists of a helpdesk for veterinarians and farmers (telephone and website), pathology and laboratory service, and herd visits, combined with specific disease surveillance and data analysis (figure 1). Specific serological surveillance is done for Pseudo Rabies (PR), Classical Swine Fever (CSF), Swine Vesicular Disease (SVD), Salmonella, and Brucellosis in commercial pig farms and PR, CSF, SVD, CSF, Foot and Mouth Disease and Trichinella in wild boar. Results will be shown at the ESPHM

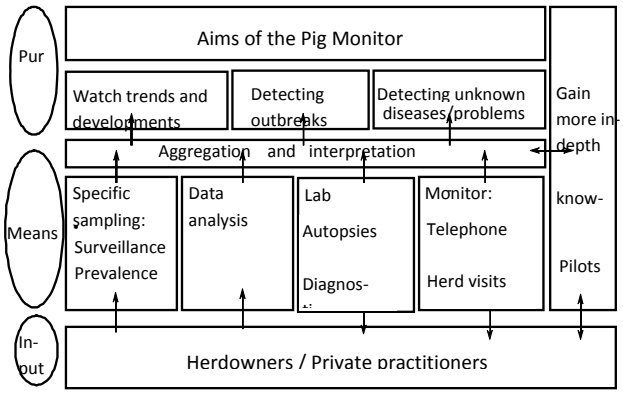

Fig. 1. Schematic representation of the system.

2. Surveillance on pig health (farm level)

To gain more insight in the health status of the pigs on each individual farm or on a chain of farms (breeders, multipliers, fattening pigs) the GD-AHS and TOPIGS have developed a monitoring system for PRRSv, APP, M, hyo, Brachyspira spp, Lawsonia spp and Salmonella. A lot of practices as well as feed companies have their own comparable system. So Dutch pig farmers are able to give some information about the health status of their pigs

3. Quality assurance system of pig production

In 1992 the Dutch Livestock Board started a market oriented, accredited quality system for the meat production chain, based on HACCP, EU and Dutch legislation and guidelines for GMP. Details of the system will be shown at the ESPHM. Farms are inspected every year for animal health, animal welfare, hygiene, feed & water, food safety, certified suppliers (e.g. feed, vets, transporters) and monitored for forbidden drugs. Nowadays both unions of pigfarmers have their own QA system, but the goals of both systems are the same.

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QUALITY ASSURANCE IN SPANISH PIG PRODUCTION

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Introduction

Spain is the second largest pig producer in Europe (17% of the total) after Germany and currently is facing a challenging situation, as many other countries in the EU.

During last years a deep crisis is affecting the sector with high prices of the feed and low prices of the pork, leaving little or even negative yearly margin to producers. Furthermore, sows' census have decreased after the peak of 2006 (-1.2 % sows 08/09)[i] but this is not fully reflected already in meat production since total census increased (+4.8 % 08/09[ii]). Cataluña, Aragón y Castilla y León are the three main productive regions representing more than the 50 % of the production, most of it arranged under big integration companies. A remark must be stressed in the Iberian pig sector where census decreased 62 % (07/09) in a situation that has been compared to real state crash. Overall, our meat self-supply rate has increased up to 137 % (04/08[iii]).

Under these circumstances, the Spanish pig sector has to face the uncertainty of prices (forecast -0.8 % this quarter), while keeping its competitiveness in terms of internal sector requirements (cost of production, carcass quality) or regulatory requirements (environment, food safety, welfare)

Health and productive performance

Overall average productivity, based on different sources consultation[iv], is slightly over 24 piglets / sow / year, but the tendency is to a clear improvement, explained by the general use of top genetics, management and health improvement. PRRS keep on being the main health problem affecting reproduction and some skepticism is growing-up regarding the use of certain vaccination programs. Pre weaning mortality is another issue where improvement is desired, and reasonably will be achieved.

Nursery and growing-finishing shows a stable / tending to improvement situation. Health situation is the best in the latest years with PRC and enteric diseases under control. The increasing use of circovirus vaccines is controlling the presence of secondary diseases as well. All of this is improving both slaughter weight (1.6 %) and FE (2.1 %)[v]. Finally, has to be said that the national plan for Aujezsky's Disease eradication is working very well with most of the municipalities with 0% prevalence; without date defined, stop vaccination is coming closer.

Environment

Currently the most growing issue affecting farmers. Spain is developing royal decrees based on EU Directives, but regions has the ability of hardening national regulations.

Besides of good agricultural practices, made compulsory for many municipalities, there is a new figure called Integrated Environmental Permit that is substituting all of the prior permits. It is linked to the IPPC (Integrated Pollution Prevention and Control EU Directive) permit and depending on the region and the size of the farm, has to be renewed every 2-8 years, asking for the implementation of the best available techniques throught the production chain (from housing to spreading, including nutrition and storaging). It is aimed to abate ammonia emissions and save water and energy during the production process.

Green house gases emissions (methane are affecting pig producers as well from 2009. A new royal decree promoting the burning of methane and its utilization for heating from pig slurry lagoon. This regulation will be developed both under the setting up of indivual biodigestores in farms or throughout centralized biogas plants.

Food safety

The last EFSA study of salmonella prevalence in Europe showed the highest prevalence in slaughter pigs in Spain 29 % with the same main serotypes present (Typhymurium, Derby and Rissen). Spain developed a National regulation (Reglamento 2160/2003) defining the objectives for reduction, the timing and the national control programs, to be finalized 2011/ 2012. Beyond that those requirements, many companies (big producers, co-ops and associations) are implementing control programs to be ready before being mandatory

or look for quality labeling in its production chain.

Welfare

For many producers, 2013 seemed to be faraway and never to come. Picture regarding this issue is offering three very different scenarios, professional producers (normally big or linked to integrations) that have changed already to grouped sows, medium farmers that because of his financial situation are not able to face the investments to fulfill the regulations, despite they are willing to stay in the business, and small to medium farmers that because of age, situation of the farm (close to villages and prospectives of the business), simply will stand while possible. It is difficult to quantify the percentage of farms that have already moved to group sows. Personal communications will rank the farms moved between 20/40 % currently.
Finally, regarding castration, one of the most controversial and burning issues in EU right now, it must be said that most of the males are boars (80 %) with a slaughter weight around 105 Kg. Only heavy pigs for Serrano ham or Iberians are castrated. For them, different immunocastration programas are being tested under commercial conditions.

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LABORATORIAL APPROACH TO THE DIAGNOSIS OF PIG DISEASES: A PERSONAL OVERVIEW

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Introduction

Diagnosis is, in a broad concept, the identification of the nature of anything, either by analytical methods or processes of elimination. Diagnosis is used in many different disciplines, with slightly different implementations on the application of logic and experience, to determine the cause and effect relationships. In the most specific medicine sense, diagnosis includes to determine the causes of symptoms, mitigations for problems, or solutions to issues. All efforts focused on identifying the nature of problems (understood mainly as diseases or poor-production scenarios in the case of pig health and production) and the establishment of curative or preventive solutions are, by definition, intrinsic components of the diagnostic procedure. Therefore, the pig veterinarian working under field conditions represents, in essence, a diagnostician.

Given the definition of diagnosis, it is evident that the diagnostic work starts as soon as the veterinarian visits the farm and tries to establish all the components of the problem. Sometimes field veterinarians rely their diagnosis mainly (or sometimes almost exclusively) on the results obtained after analysis given by a Veterinary Diagnostic Laboratory (VDL) (Martineau, 2005). It is paramount at this time to quote Gardner and Blanchard (2006) in the sense that even diagnostic laboratories can help identifying agents potentially involved in a disease outbreak or poor-production problem, the importance of pathogens relative to other host, management, and environmental factors must be determined by the submitting veterinarian. In other words, laboratorial diagnosis is an additional tool within the diagnostic arsenal available to the field veterinarian. Moreover, and depending on the information given to the VDL by the submitting veterinarian, the outcome of the VDL is just an analysis result that must be carefully interpreted in the context of the global farm scenario. As a result of experience, this author would like to emphasize (and criticize) the sharp "microbiologistic" trend of pig veterinarians. It is true that pig medicine mainly demands a population diagnostic approach, so, infectious/contagious diseases are perceived as the most important ones. In other words, the usual first question by pig veterinarians in front of a given disease problem is "which is the causal pathogen?". One must admit that, even pathogen participation is very frequently present in disease problems, a number of times the infectious agent reflects an added factor to a non-infectious primary cause or triggering factor. Importantly, the counteraction of such non-infectious primary cause or triggering event may represent the solution of the disease scenario, while the specific control of the found pathogen might represent only a temporary solution. It is clear, therefore, that pig veterinarians face usually with a complex, multifactorial scenario in which a number of elements may interact at the same time. In this context, the aid of laboratorial testing can be of great help, and the intention of this paper is to highlight basic but important aspects of diagnostic procedures as well as to discuss on current and new laboratorial approaches to help establishing the final diagnosis by field pig veterinarians.

The diagnostic approach and the use of laboratorial testing

Pig veterinarians use diagnostic tests to investigate on health, productivity and reproductive status in individual animals and herds. Besides corresponding action farms (visit, clinical history and farm background assessment, revision of production records, on-farm epidemiological and risk factor investigations, necropsies, etc.), tests that involve the submission of samples to a VDL might be used. These laboratorial tests are mainly used to (Gardner and Blanchard, 2006):

- Detect pathogens or toxins that are responsible (or not) for disease outbreaks or suboptimal production
- Evaluate the infection/exposure status of individual pigs
- Determine if a herd was infected with or exposed to a pathogen and, if so, which age or production groups were affected
- Estimate the percentage of herd or pigs with antibodies to an infectious agent
- Monitor a herd's serological response to vaccination

- Monitor the progress and success of disease control or eradication programs

The abovementioned diagnostic purposes are aimed to answer the fundamental question: "what am I interested to confirm or rule out?". To sample correctly and request for the adequate tests will help the veterinarian obtaining the answer. Importantly, such question must be formulated in a way that the VDL can really answer it. Laboratorial tests may confirm whether a certain infectious agent or toxin is present in a given sample or samples in a pig or group of pigs, but they cannot establish if this agent or toxin is the major one involved in a particular herd problem or if the treatment strategy against the organism will solve the situation.

Several criteria should be applied to decide which laboratorial test to use in each particular disease problem, such as the cost and the rapidity in which the test is performed and the results communicated. However, other criteria should also be considered (Segalés, 2005):

- Own features of the test (sensitivity, specificity, predictive values and accuracy). A cheap, rapid, easy-to-do test with 100% sensitivity and 100% specificity does not exist. All tests are imperfect
- Resources and abilities of the laboratory: "all laboratories cannot test all". This situation claims for a really deep contact and relationship between the veterinarian and the laboratory
- Do not test for something with doubtful interpretation (i.e., serology for a certain infectious agent of a single pig, PCR of a single pig, etc.)

To choose one or another laboratorial test must be based on the advantages and disadvantages that techniques offer, which are crucial for the adequate interpretation of results. It is noteworthy that in a significant number of cases, the pig veterinarian is not aware on the basic knowledge on laboratorial tests (Table 1). This situation implies that the wide availability of laboratory tests is parallel with certain lack of information regarding which test should be used in each particular situation. Pig veterinarians must know also strengths and weaknesses of laboratorial tests (Segalés, 2005) in order to address the fundamental question raised above. As an example, sometimes less sensitive techniques (such as the immunofluorescence antibody test in frozen tissues to detect a pathogen) are more useful to determine the cause of a clinical problem than more sensitive and specific techniques (such as a polymerase chain reaction (PCR) method).

Highly sensitive tests are not necessarily the best option to diagnose a clinical disease problem. New high-tech modern techniques (especially molecular biology techniques) have open new and challenging scenarios on diagnostic capabilities. Molecular techniques, in comparison with classical microbiological methods, have many advantages in the sense that they are becoming less expensive, more rapid and sensitive, have high throughput, do not require viable agent and offer more portability of the generated data (Foxman et al., 2005). However, a number of these techniques are still poorly available in VDLs across Europe. Moreover, in some cases, the availability of the technique is no accompanied by the sufficient expertise to exploit adequately the outcome of the technique. An example would be sequencing as a molecular tool for epidemiological investigations. The capability of sequencing does not imply the availability of a diagnostic test unless proper handling and interpretation of bioinformatic sound data is feasible (Olvera et al., 2010). At the end, and even the arsenal of diagnostic tools is expanding (Belák, 2007), the rational attitude of the pig veterinarian regarding laboratorial analyses is to request those tests for the most probable differential diagnoses, and not for all of the possibilities, including the most remote ones.

Missing points in the laboratorial testing framework

In-depth reports or publications concerning methodology of clinical data collection and interpretation are very limited. Moreover, a paramount issue as pig and sample selection to be laboratorially investigated is a rather neglected matter among professionals. One must assume that reliability and confidence of a laboratorial technique does not depend exclusively on the own features of the test, but importantly on the quality and real representativity of the tested sample. Pig veterinarians must assume that they have a very important impact on the final result of a laboratorial test just by proper sample selection and submission to the VDL. To avoid pitfalls, a deep veterinarian-VDL contact is desired.

Here we are at the framework of the 2nd European Symposium on Porcine Health and Management. Therefore, a significant number of countries are represented. When looking at the VDL capabilities and laboratorial test availability in different countries, significant differences are detected. This subject has a

key importance in regards the routinely diagnostic work for pig veterinarians across Europe. Regretly, final diagnostic capabilities of pig veterinarians depend, somewhat, on where are you living.

There are a couple of other important issues we have to face in Europe: lack of transboundary test harmonization and lack of a VDL accreditation.

It is assumed that VDLs do efforts in order to validate laboratorial techniques. Validation imply "establishing documented evidence which provides a high degree of assurance that a specific process (here the laboratory test) will consistently produce a product (here the result of the test) meeting its pre-determined specifications and quality attributes" (FDA's Guide on General Principles of Validation, 1987). International validation and standardization of the diagnostic assays is very important today. National and international authorities require rigorous proof that the assays, used in various laboratories, are as reliable as possible and give identical results. International agencies like the OIE (World Organization for Animal Health), the Joint FAO/IAEA (Food and Agricultural Organization / International Atomic Energy Agency Division), national research institutions and commercial companies make large efforts to agree on international standardization (Belák, 2007). The OIE regularly publishes standards for the validation of diagnostic assays (http://www.oie.int). However, such validation protocols are given for tests corresponding to a limited number of diseases. Therefore, validation of all routine laboratorial tests is not systematic in public and private VDLs. Moreover, test harmonization and ring trials (besides those applying to diseases of the OIE list) among European laboratories is rarely performed. In addition, there is not a supra-national body that accreditates (certification of competency, authority, and credibility) VDLs.

In North-America, the American Association of Veterinary Diagnostic Laboratories (AAVLD) (http://www.aavld.org) is in charge of an accreditation program. Its purpose is to accredit public veterinary diagnostic laboratories in North America relative to technical and operational competence compatible with appropriate standards, and to provide an administrative assessment. A number of objectives must be met in the accreditation program:

• To provide a mechanism for objectively accrediting veterinary diagnostic laboratories
• To continuously emphasize the importance of excellence in veterinary diagnostic service
• To periodically evaluate and modify the accreditation process
• To keep laboratories cognizant of current technological advances in diagnostic veterinary medicine
• To keep laboratories informed of the impact of legislative mandates and other regulatory actions
• To promote adequate training of specialists in diagnostic veterinary medicine
• To encourage hiring of dedicated and innovative diagnosticians with appropriate training and experience
• To encourage acquisition and maintenance of facilities suitable and adequate to provide quality services
• To promote appropriate quality system programs
• To assist laboratories to meet or exceed the standards of the OIE

Conclusions

Diagnostic procedures are key elements in pig health and management. This is a field of constant evolution and even the basis relies on a good and sound clinical investigation, laboratorial testing has represented a technical revolution during last 10 years. PCRs were just used for research not so time ago and today have become one of the most broadly used test in VDLs. The same will probably happen (it is already happening!) with other technological platforms such as quantitative real-time PCR, sequencing with phylogenetic analysis and microarrays. In all cases, appropriate expertise is necessary and VDLs are of key importance in introducing these analyses. On the other hand, pig veterinarians must have the sufficient knowledge on the laboratorial tests to be used. Their understanding will provide a more critic and reasonable interpretation of results. At the end, these laboratorial tests do not provide a diagnosis, but important information to establish it by the submitting veterinarian. Finally, VDLs must also move a step forward in order to fully validate their tests as well as to create a pan-European accreditation system that guarantees quality assurance and control all around the continent.

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Table 1. Characteristics of each laboratorial technique: what is it detected and what is it not detected (adapted from Segalés, 2005).

Laboratorial technique	Basis
Bacterial isolation	Ability of bacteria in growing in a base of solid or liquid nutritional elements
Viral isolation	Ability of viruses in growing in certain cell lines; detection of the precise agent is based on IF or immunoperoxidase on the cell culture
Serotyping	Differentiation between strains (serotypes) of microorganisms that have differences in the antigenic composition of a certain structure such as the cell wall or flagella by means of a panel of antibodies
Immunofluorescence (IF)	Detection of pathogen antigen/s based in a specific antibody conjugated with a fluorocrome; usual detection in frozen tissues
Immunohistochemistry (IHC)	Detection of pathogen antigen/s based in a specific antibody; usual detection in formalin-fixed, paraffin-embedded tissues
In situ hybridization (ISH) or fluorescent ISH (FISH)	Detection of a certain genome sequence of the pathogen based in a specific hybridisation probe; usual detection in formalin-fixed, paraffin-embedded tissues
Polymerase chain reaction (PCR)	Exponential replication of a target double stranded DNA based on the use of two primers (reverse and forward) and a DNA polymerase in a different number of amplification cycles
Nested PCR	It is a double PCR method. Once the first PCR has been accomplished, a second set of primers that detects part of the sequence obtained in the first PCR product are used to perform the second PCR
RT-PCR	Exponential replication of a complementary DNA obtained by reverse transcription of a target RNA
Quantitative PCR or RT-PCR	Quantification of starting amounts of DNA, cDNA, or RNA templates. It is based on the detection of a fluorescent reporter molecule that increases as PCR product accumulates with each cycle of amplification. Fluorescent reporter molecules include dyes that bind double-stranded DNA or sequence–specific probes
ERIC-PCR	Fingerprint of the genome by examining strain-specific patterns obtained from PCR amplification of the ERIC sequences, which are 126-bp elements that contain a highly conserved extragenic regions of the bacterial genome
Sequencing	Determination of the nucleotide sequence of the genome or part of it, usually from a PCR product or clones
Multilocus sequence typing (MLST)	Involves PCR amplification followed by DNA sequencing of a set of genes that allow characterizing strains by their unique allelic profiles
Microarrays	Arrayed series of a number (can be thousands) of microscopic spots of DNA oligonucleotides that are used to hybridize target DNA or RNA. Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the sample tested
ELISA test	Detection of antibodies by binding them to protein/s of a given pathogen, which are coating 96-well plates (antigen specific antibody ELISA), or detection of antigen in specific antibody coated 96-well plates (antigen detection ELISA). Technique based in immune-enzymatic methods.
Virus-neutralization test	Detection of antibodies that are able to neutralise viral multiplication in cell culture
Complement fixation test (CFT)	Detection of antibodies to a given pathogen in a system including complement, which only reacts in the presence of antibody-antigen complexes. The indicator system used in this technique is sheep red blood cells

Hemagglutination inhibition test	A known quantity of antigen is added to the problem serum prior to the addition of a red cell suspension. Reaction result is expressed as the smallest amount of antigen which causes complete inhibition of hemagglutination
Histopathology	Microscopic examination of fixed tissues (usually 10% formalin) stained with standard dyes (usually haematoxylin and eosin)

MONITORING VIA "MEAT JUICE SEROLOGY"

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New paradigms need new approaches

The traditional meat inspection procedures at slaughter, focussing at identifying health risks for humans by condemning carcasses and organs that show pathological signs has resulted in the eradication and/or control of most "classical" food-borne threats to human health such as tuberculosis, brucellosis and tape worms. However, the still high number of food-borne diseases in humans such as salmonellosis, yersinioses and the health risks due to chemical or pharmaceutical residues (none of them leaves pathological signs recognisable at the slaughter line by traditional meat inspection procedures) prove that the traditional ante- and post-mortem inspection of single carcasses as end product inspection is not able to sufficiently prevent and control the risks of today (3).

Therefore the European Commission has issued the so-called "Hygiene Package", with Regulation 852/2004/EC ("on the hygiene of foodstuffs"), Regulation 853/2004/EC ("laying down specific hygiene rules for food of animal origin") and Regulation 854/2004/EC ("laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption and the principles of the food chain information"), which describes the transition from the traditional to the risk-based meat inspection. These Regulations in combination with the Direktive 2003/99/EC and the Regulation (EC) 2061/2003 (the "Zoonoses-Regulations", the Regulation (EC) 2075/2005 ("specific rules on official controls for Trichinella in meat"), Regulation 1244/2007/EC ("requirements for a visual meat inspection") and with different National Regulations, e.g. the German Regulation on the monitoring and control of Salmonella in pigs and pork, result in an overall system, which observes the entire food chain "from stable to table". This change of the food safety paradigm from end product inspection to process optimisation along the food chain aimes at the continuous improvement of food safety, animal health and animal welfare (1). Another paradigm shift is that the EU legislative does not any longer prescribe exactly the inspection procedures for all Member States in the same way, but defines the common food safety goals.

Thus, each EU-Member State has to develop its own and specific risk profile and the ways of controlling and managing the risks in question (4).

The new approach that is needed to develop feasible procedures for the new paradigm demands for envolving the animal production into the food safety concept more knowledge on the animal health and drug use in the pig herds that supply a slaughterhouse. To achive this goal, the legally required "food chain information" (Reg. [EC] 853/2004) is to enable the official veterinarian to assess the risk level for slaughter batches of the supplying pig herds, which is the basis for her/his decision on the intensity of the meat inspection. The principle is: pig carcasses from herds that can be regarded as "high health status and low residue risk" can be inspected "visually" and pig carcasses from herds that must be regarded as "low health status and high residue risk" must be inspected "in depth according to the risk in question".

Components of the food chain information are data on the feed origin, the animal health (especially notifiable and food safety relevant diseases), and the antibiotic usage per pig supplying herd. Additionally to these data collected by the herd owner, data are to be generated through continuous monitoring programmes focussing on zoonoses. This means that national, regional and food chain specific programmes are needed. This clearly implies that serological monitoring systems have to be implemented for generating meaningful information about the occurrence or absence of zoonotic infections in pig herds. At present, there are only monitoring systems for the detection of antibodies against Salmonella.

This paper explores opportunities and possibilities of a "multi-diagnostic" serological monitoring for identifying and quantifying as many health risks carried by slaughter pigs into the food chain to humans as possible. The existing Salmonella control programmes are mostly using meat juice as specimen for detecting Salmonella antibodies. This and the fact that collecting meat juice is logistically easier to handle than collecting blood serum in the herd and at the slaughter line has led to the idea to test the usability of meat juice for detecting also other infections than Salmonella

in pigs. To achieve a high efficiency of taking and testing samples for antibodies, the samples should not only tested serologically for zoonotic pathogens, but also for pathogens causing notifiable diseases as well as for pathogens of production diseases of pigs.

Testing the usability of meat juice

First step: Taking into consideration the needs for a meaningful serological monitoring using random samples per pig herd for defining the zoonosis and pig disease risk herd-profile, a set of serological tests was selected, which provides results with relevance for human health (measuring antibodies of zoonotic pathogens) as well as for pig herd health (measuring antibodies of infectious pathogens for pigs, both notifiable and non-notifiable diseases). For our study, we decided to start with detecting antibodies against the infectious agents that are of interest for three interest groups:

1) Salmonellla, Trichinella, Toxoplasma, Yersinia and Mycobacteria, which are of interest for the food chain operators and the official veterinarians responsible for food safety,

2) Classical Swine Fever and Pseudorabies, which are of interest for the official veterinarians responsible for the prevention and control of notifiable animal diseases, and

3) Mycoplasma hyopneumoniae as well as Influenza A, which are of interest for the pig producers.

Second step: To evaluate the general usability of meat juice as specimen for the currently available ELISA tests for pigs, which are licensed for blood serum, several test runs with lower dilutions than prescribed for blood serum were conducted to find the appropriate dilution for meat juice. The dilution of the meat juice 10 times less than that of blood serum turned out to produce the most comparable results of meat juice testing to the results of the serum testing.

Third step: Altogether, from 328 pigs a blood serum sample (taken at the point of bleeding the animals after stunning) and a meat sample from the diaphragm pillar of exactly the same pigs/cacasses (taken at the point of meat inspection) were collected. It was assured by additional tattooing of the pigs at bleeding that there were 328 specimen pairs (serum and meat juice) that both samples were unmistakably from the same animal.
After freezing and thawing of the meat pieces for producing the meat juice, all samples were tested with seven different ELISA-tests taking the 10 times lower dilution of the meat juices into consideration.

Results

Apart from the results concerning the usability of meat juice instead of blood serum, Table 1 presents the frequency of the occurrence of the tested antibodies (percentage of positive samples) in the pig herds the samples came from.
Although the number of positiv meat juices was in all cases slightly lower than the number of positive serum samples, the closeness of agreement (positive and negative results included into the calculation of the agreement) is not lower than 84%. The tests with the lowest closeness of agreement (Influenza A and Mycoplasma hyopneumoniae) are those that have not been developed, validated and licensed for meat juice.

Tab. 1: ELISA test results from blood serum and meat juice of 328 slaughter pigs

Relevance for	ELISA test kits for	Blood serum: Positive	Meat juice: Positive	Closeness of agreement (all samples)
Human health	*Salmonella spp.*	17% (55)	14% (45)	94%
	Yersinia enterocolitica	71% (233)	69% (227)	94%
	Toxoplasma gondii	3% (9)	2% (5)	96%
	Trichinella spiralis*	0% (0)	0% (0)	100%
Herd Health	*Mycoplasma hyopneimoniae*	57% (188)	50% (164)	86%
	Influenza A (H1N1)	31% (102)	17% (55)	84%
	Influenza A (H3N2)	14% (45)	8% (25)	85%
Surveying notifiable diseases	Classical swine fever	still to be tested		
	Pseudorabies			

*all Trichinella positive control sera provided by the National Reference Laboratory for Trichinella were clearly identified

Discussion & Conclusions

The results suggest that the closeness of agreement between the measured serum and meat juice antibody concentrations is sufficient for the decision to pursue the development of a multi-diagnostic “meat juice serology” for pig herds. It is necessary to remind of the fact that any serological monitoring, i.e. also a “meat juice serology”, is not thought to deliver single animal answers, but to produce **herd health profiles** with semiquantitative estimations of the herd prevalence in question. Even with a an agreement between serum and meat juice of 85%, a classification of herds in terms of relative risk levels, is possible. Creating a system of serological herd profiles for the most important infections, even on the basis of only limited sample sizes per herd, provides the opportunity for introducing benchmarking systems and for a targeted decision making both for food safety and any animal healh improvements.

Apart from these opportunities, the major advantage of the suggested approach is that this kind of multi-diagnostic monitoring addresses three groups of stakeholders: the consumers, the veterinary authorities, and the pig producers. Offering all three groups continuous information that serves their specific interests will provide the benefit to share the costs of such monitoring systems: the food producers pay part of the costs for the information on zoonotic diseases, the government pays part of the costs for the information to improve the surveillance of notifiable diseases, and the pig producers cover part of the costs for getting continuous information about the infectious status of their pig herds, especially about the diseases that impair the profitability of their pig herds.

These results and considerations about the new appraoch have led to intensive cooperations of our two university institutes (the Field Station for Epidemiology and the Institute of Food Quality and Food Safety of the University of Veterinary Medicine Hannover) with diagnostic companies, state laboratories for diagnosing notifiable diseases, and slaughterhouses a) for improving the specificity and sensitivity of the planned tests that are to be included into the “meat juice serology”, and b) for adding further causative agents to the panel of tests such as *Mycobacterium avium ssp. paratuberculosis* (MAP), *Campylobacter spp., Actinobacillus pleuropneumoniae,* and *Pasteurella multocida toxin D+.* We will report about the outcome of these cooperations at the 3rd ESPHM in Helsinki in 2011.

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TEST VALIDATION IN INFECTIOUS DISEASES: A PERMANENT CHALLENGE TO FIT WITH CLINICIAN AND EPIDEMIOLOGIST NEEDS

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Introduction

The validation of a test is the evaluation of a process dedicated to determine whether the test is fitting to a particular use or not. A validated test results in making certain the presence of a particular *analyte* (e.g an antibody or a part of microorganism) and enables to draw a prediction about the status of the subject submitted to the test.
For infectious disease diagnostic tests, the identity and definition of the criteria required for test validation are elusive, and the process leading to a validated test is not fully standardised. Furthermore, for a single pathogen there are numerous and very different needs for using tests in the field, that often require specific validation.

To what questions the clinician or the epidemiologist may want to get an answer?
Disease diagnostic
Animal or herd status: Infected / Uninfected
Many aspects of management in infected herds (maternal derived antibodies, viraemia, active immunity) and links to vaccination (vaccinated / not-vaccinated – i.e. DIVA, interferences, protection), clinical observations or treatment efficacy.

Definitions

- Analytical sensitivity of the test is the smallest detectable amount of the analyte that can be detected
- Analytical specificity is the degree to which the test does not cross-react with other analytes. These parameters are different from diagnostic sensitivity and specificity as defined below.
- Diagnostic sensitivity is the proportion of known infected reference animals that test positive: infected animals that test negative are considered as “false negative” results.
- Diagnostic specificity is the proportion of uninfected reference animals that test negative in the test: uninfected reference animals that test positive are considered as “false positive” results.

Because of the numerous variables that must be accounted for, at least 300 reference samples from known-infected animals, and not fewer than 1,000 samples from known-uninfected animals, should be included to determine initial estimates of Diagnostic sensitivity and Diagnostic-specificity, respectively.

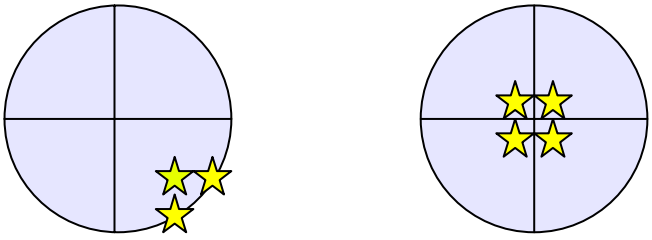
- Repeatability and reproducibility are estimates of the precision of the test.
Although there is a continuum of situations between repeatability and reproducibility, it is commonly considered that reproducibility is the amount of results of samples tested both in different laboratories as positive or negative.

- Accuracy is the amount of agreement between a found test value and the expected test value for an analyte attesting a standard sample of known activity (titre for example)
A test system may be precise, but not accurate, if the test results do not match with the expected value for the standard.

Diagram 1: Difference between precision and accuracy

Precision

Accurracy



Principles of test validation

1 It is an **experimental process** who needs an experience plan

2 It is a **limited range process**

- reference samples have to be representative for field populations which remains a big challenge!
- A quantitative test is valid within a quantification zone (quantitative PCV2 ELISA for instance)

3- It is a **relative process** :

- Diagnostic sensitivity and specificity are calculated with a reference population (PRRS for instance)
- Predictive values: an estimate of the prevalence in the target population is necessary for calculation of the predictive values of positive (PV+) or negative (PV-) test results.

4- It is a **continuously improving** process

- Confidence in the test improves with the number of tests performed
- The process is time dependent (stability) : Fidelity of the test is important to both clinicians or epidemiologists and laboratory diagnosticians
- The process also depends on the actual prevalence of infection : predictive positive value decreases in parallel with infection prevalence in the population.

The overall validity of the response even using a validated test is depending on:

The sampling procedures including the choice of animal(s), date of sampling, type of samples, number of samples, potential sample contamination, inhibitors (PCR tests, antibiotics…)

The course of infection (biological data like duration of bacteraemia/viraemia, range of sero-conversion (IgG, IgM), etc), and links to clinical/sub-clinical findings.

Conclusions

For the validation of a test, the selection of the reference populations is the most critical factor. It is not surprising when reviewing the literature to sometimes find a wide range of values and conclusions for sensitivity and specificity for a single test !....

For Interpretation: test results are valuable only if the inferences made from them are accurate.
Laboratory needs validated tests and Clinicians need to use proper diagnostic strategies to keep these tests providing interpretable results. The interaction between clinicians and lab diagnosticians remains essential for this purpose.

Relying on a single test to fit with all different kind of needs will remain a challenge.

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VALIDATION WITHOUT GOLD STANDARD

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The World Organisation for Animal Health (OIE) has adopted epidemiological principles for establishing diagnostic sensitivity (Se) and specificity (Sp) as performance characteristics of diagnostic tests (OIE, 2009). The conventional approach requires that animals are sampled from the target population and subjected to a reliable reference test ("gold standard"), allowing Se and Sp being estimated relative to the gold standard (Greiner and Gardner, 2000).

However, it has been recognized that the lack of a gold standard is a common limitation in validation studies. Using the conventional approach, it is impossible to demonstrate that the new test has better Se or Sp than the gold standard test. So-called latent class models have been developed to overcome this problem (Hui and Walter, 1982; Enoe et al., 2000).

In this presentation I discuss possible study designs applicable in pig health for estimating Se and Sp of a diagnostic test without gold standard. The underlying assumptions and requirements will be explained in the given application context. I will illustrate using an example of diagnostic tests for detection of *Toxoplasma gondii* infection.

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QUALITATIVE AND QUANTITATIVE DISTRIBUTION OF PCV2 IN WILD BOARS AND DOMESTIC PIGS

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Introduction

Porcine circovirus type 2 (PCV2) is considered the essential infectious agent of a series of diseases in swine, collectively termed porcine circovirus diseases (PCVD). PCV2 has also been detected in wild boars from several countries by serological studies, PCV2 antigen and DNA detection, indicating that PCV2 circulates at a high rate among the wild boar populations in Europe. PMWS reports in wild boars are scarce, however. In domestic pigs, the high incidence of subclinical infection and the stability of the virus complicate diagnostics and appraisal of PCV2 infection and PCVD. Diagnostics and prognosis based just on serology or conventional PCR overestimate the risk of developing PCVD. However, several studies have provided evidence for the association between PCVD and the PCV2 load in serum or tissues of pigs (Brunborg et al., 2004; Olvera et al., 2004; Segales et al., 2005). Neither the PMWS status nor the quantitative load of PCV2 has been documented comprehensively in wild boar populations. We hypothesize that the countrywide prevalence of PCV2-infected wild boars is high with low incidence of PCVD, and that the discrepancy between both is associated with low loads of PCV2.

Material & Methods

Samples from different tissues of 531 wild boars from 46 different hunting grounds and from 308 apparently healthy and 40 wasting (rejected at slaughter) domestic pigs from 48 provenances located in the same greater areas as the wild boars were analysed. PCV2-specific DNA was detected independently by nested PCR (nPCR) and by quantitative realtime PCR (qPCR). PCV2-specific tissue alterations were examined by histological (HE) and immunohistochemical (IHC) standard procedures. Pigs were classified as PCVD-affected (moderate to severe lymphocyte depletion and granulomatous inflammation of the lymphoid tissue, and moderate to high amounts of PCV2 genome equivalents [at least $5x10^6$ copies of PCV2 DNA/µg extracted DNA in at least one of the tissues]), subclinically infected with PCV2 (no or only slight lymphoid lesions, and a lower amount of viral genome copies), and non-PCVD (no lymphoid lesions and negative in qPCR) (Segales et al., 2005). The threshold of $5x10^6$ PCV2 DNA copies per µg of extracted DNA corresponds to 10^7 copies of other studies (e.g. Harding, 2008).

Results

PCV2 prevalence, determined by nested PCR, was 63.1% in wild boars and 100% in domestic pigs ($p<0.001$). Only 45.4% of the wild boars, but 98.8% of the domestic pigs were tested positive for PCV2 by qPCR. PCV2 loads differed significantly ($p<0.001$) between wild boars and domestic pigs. The average viral load was $10^{2.8}$ in tissues of wild boars and $10^{4.2}$ in those of domestic pigs. None of the 349 wild boars did definitely show clinical symptoms of PCVD, but one of them (0.3%) met the criteria for PCVD. Based on the same classification, 8.7% of the domestic pigs from 15 of the 48 provenances were found to be PCVD-affected. None of the wild boars and 7.1% of the domestic pigs had PCV2 loads above $5x10^6$ DNA copies per µg of DNA. The number of PCVD-affected and non-affected pigs did not differ between pigs slaughtered and wasting pigs that were rejected at slaughter.

Discussion & Conclusions

In conclusion, this is the first study that describes the nationwide distribution of PCV2 in wild boars and domestic pigs qualitatively and quantitatively. As the level of PCV2 load appears to be the critical step in the development of severe PCVD, qPCR proved to be well suited for the evaluation of PCV2 infection and PCVD in wild boar and domestic pig populations.

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ORF2 AND ORF3 GENOTYPES OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) IN WILD BOARS AND DOMESTIC PIGS IN GERMANY

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Introduction

Porcine circovirus 2 (PCV2), the essential infectious agent in PCVD (porcine circovirus diseases) circulates at high rates among domestic pig and wild boar populations. Wild boars may be viremic and shed the virus with excretions and secretions, and thus serve as a reservoir for domestic pig PCV2 infection. We hypothesize that PCV2 strains circulating in wild boars and in domestic pigs are different and thus, partially independent. To prove this hypothesis, the distribution of ORF2 and ORF3 genotypes of PCV2 within wild boars and domestic pigs from overlapping greater areas of Germany was investigated.

Material & Methods

Samples from 40 wild boars from 17 different hunting grounds and tissue samples of 60 apparently healthy domestic pigs from 18 provenances located in the same greater areas were analysed. PCV2-specific DNA was amplified by nested PCR (nPCR). PCV2 genotypes were classified based on ORF2 sequences as described by Olvera et al. (2007) and Segalés et al. (2008). ORF2 and ORF3 genotypes were detected by pyrosequencing on a PyroMark ID (Biotage, Sweden) system. Genotypes were compared with PCV2 sequences from the Genbank database (520 entries).

Results

Two ORF2 and nine ORF3 genotypes were detected in twelve combinations, with significant differences between wild boars and domestic pigs. PCV2-2b was the most common genotype in both species. Almost 60% of the infected wild boars but only 4.8% of the infected domestic pigs carried the PCV2-2a subtype. ORF3 genotype frequencies differed also significantly between wild boars and domestic pigs. Some ORF3 genotypes were detected in domestic pigs only, others exclusively in wild boars. The genotypes of domestic pig PCV2 samples were dominated throughout the country by 2b/ORF3-1 and 2b/ORF3-3 types. PCV2 genotypes of the wild boars were, however, more heterogeneous than those of domestic pigs. Two different PCV2 genotypes in one animal have been isolated from 27% and 4% of wild boars and domestic pigs, respectively ($P<0.001$), and at least two different PCV2 genotypes have been isolated from 90% of hunting grounds and 50% of domestic pig provenances. The five Genbank database PCV2 sequences from German domestic pigs (from 1999/2000) differed significantly from the "domestic" sequences of the present study (2004/2007) and from 124 sequences obtained from different European countries. However, European and the "domestic" sequences of the present study were in good agreement. The six Genbank sequences of European wild boar isolates agree with the major genotypes of the wild boars of the present study. Some of the wild boar derived genotypes are not present or very rare among "domestic" and wild boar Genbank sequences worldwide.

Discussion & Conclusions

Differences in "domestic" PCV2 genotypes of the present study and Genbank entries from Germany may indicate the general switch from PCV2-2a to genotype PCV2-2b after the year 2003 that has been generally described by Dupont et al. (2008). Differences and conformity of PCV2 genotypes derived from wild boars and domestic pigs, either from this study or from European Genbank database entries, indicate that wild boar and domestic pig PCV2 do coexist with some exchange. More than 50% of the wild boar PCV2 genotypes are extremely rare in domestic pigs in Europe (Genbank) and Germany (this study), even worldwide, inferring a certain independence of wild boar and "domestic" PCV2 genotypes. The fact that genotype 2a is prevalent in wild boars (57.5%), but rare in domestic pigs (4.8%) and that genotype 2b is the almost exclusive genotype in domestic pigs and also prevalent in wild boars, argues for the hypothesis that exchange of PCV2 between both species emerges primarily from domestic pigs to wild boars and less in the reverse direction. This hypothesis is supported by the finding that PCV2 loads of wild boars are significantly lower than those of domestic pigs.

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RETROSPECTIVE STUDY ON SWINE TORQUE TENO VIRUS GENOGROUPS 1 AND 2 AND PORCINE CIRCOVIRUS TYPE 2 COINFECTION IN ITALIAN PIG SERA

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Introduction

Torque Teno virus (TTV) is a non-enveloped, circular, single-stranded virus that has been detected in humans and animals, including pigs (1). Two swine TTV genogroups (TTV1 and TTV2) have been described (2) and the ubiquitous nature of this infection suggest that TTV have been infecting pigs for a long period of time. Moreover, recent studies suggest that swTTV could play an aetiological role in pig diseases, in particular PMWS (3). The main objective of this retrospective study is to asses whether there was a statistically significant association between swTTV and PCV2 infection.

Material & Methods

Ninety five pig sera sampled between 1990 and 2009 were used in this study (no more information about these sera was available). A maximum of 10 pig sera per year were analysed. As much as possible, were analysed two year periods at the time (Table 1). Sera were tested to detect swTTV1 and swTTV2 using specific PCR methods. Moreover, some PCR products from both swTTV genogroups were sequenced and phylogenetically analysed.

Results

Both swTTV genogroups were found in pig sera from the very first year examined (Table 1).Taking into account the whole period under study, 73 out of 95 animals (76,84%) were infected with one or the other genogroup of swTTV, while 27 out of 95 pigs (28,42%) were co-infected with both genogroups. swTTV genogroup 1 (54 out of 95, 56,84%) was more prevalent than genogroup 2 (46 out of 95, 48,42%). Moreover, 41 out of 95 animals (43,16%) were infected with PCV2 (Table 1). Moreover, 20 out of 95 animals (21,05%) were co-infected with swTTV1 and PCV2, while 27 out of 95 animals (28,42%) were co-infected with swTTV2 and PCV2 (Table 2).

Phylogenetic analyses indicated no relationship between the date of virus detection and the cluster in the distance trees.

Table 1. number of swTTV1, swTTV2 and PCV2 PCR positive pigs. n=number of analysed sera.

Year	n	TTV 1 or 2	TTV 1 and 2	TTV1	TTV2	PCV2
1990	10	4	1	3	2	0
1992	10	8	1	1	8	8
1996	5	1	0	0	1	1
1998	10	10	7	10	7	1
2000	10	10	5	8	7	5
2002	10	6	0	6	0	1
2006	10	6	0	5	1	1
2007	10	8	3	5	6	7
2008	10	10	7	8	9	9
2009	10	10	3	8	5	8
tot	95	73	27	54	46	41
%		76,84	28,42	56,84	48,42	43,16

Table 2: percentage of co-infection swTTV (1 and 2) and PCV2

%	TTV1+	TTV2+
PCV2+	21,05	28,42

Discussion & Conclusions

This study indicates that TTV genogroups have been circulating al least since 1990 in the Italian pig population.

Moreover, these results indicate a statistically significant association between swTTV2 and PCV2 infection, but not between swTTV1 and PCV2 (Fisher's exact test, $p<0.05$).

Phylogenetic analyses show no significant changes in the proportion of viral genogroups over time.

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EPIDEMIC OF ABORTION IN SOWS IN THE SECOND TRIMESTER OF PREGNANCY?

(The disappointing opportunities for retrospective investigations)

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Introduction

The Animal Health Service (AHS Deventer, The Netherlands) performs a pig health monitoring program commissioned by the Dutch Ministry of Agriculture and the Product Boards for Livestock, Meat and Eggs (PVE). Within this program, it was noticed that from July to November 2008 many abortions were reported in sows in the second trimester of pregnancy. Moreover, a remarkable number of aborted foetuses were sent to the AHS for post mortem examination. According to the local vets, the clinical picture of these abortions could be described as 'a wave of abortions spreading within sow herds'. After several months the problem faded out. The symptoms suggested that an infectious disease caused these abortions. For better understanding of the background of the problem, in the autumn of 2009 the AHS conducted a small pilot study. The aim of this study was to seek evidence for infectious (or non-infectious) causes of abortion in the second trimester of gestation. Furthermore, we compared the monitoring information from 2009 to the 2008 reports.

Materials and Method

On 6 farms with more than 5% abortions in the second trimester of gestation (between day 40 and 80) at least during one month, blood samples were collected from at least 5 sows. These samples were tested on antibodies against influenza (HI). We decided to refrain from testing for leptospirosis, because of the unreliability of the available antibody tests. Aborted foetuses from the farms as well as placentas were examined for inflammation that could indicate Chlamydophila and Coxiella (histology) or were tested for the presence of PRRSv or PCV2 (PCR), Coxiella or Chlamydophila (IHC). During a farm visit herd characteristics were registered that may be related to abortion.

Results

Initially 9 farms opted to participate in the pilot study, 3 of which could not meet with the inclusion criteria. From the other 6 herds blood samples were investigated from 42 aborting sows. All sows appeared to be seropositive for at least one serotype of PI. Seroconversion was found in 4 herds, but in no more than 50% of the sows. This was similar in 55 'control' sows that did not abort. From 15 sows we examined aborted foetuses, in which PRRSv and PCV2 were not found. In one foetus a myocarditis was established. In 6 cases also the placentas were sent with the foetuses All 6 placentas tested negative for Chlamydophila and 2 had placentitis, in which cases no Coxiella was found. In 2 placentas calcium deposits were observed, the significance of which is unclear. The clinical symptoms were not very consistent in the different herds. For instance the period during which abortions occurred differed per herd. On one farm there were sows showing too many stillbirths. Certain farm and management conditions were noticed that may be related with the occurrence of abortion like poor indoor climate, microbiologically contaminated water, bad timing of transferring sows to group housing or feeding of placentas to gilts in order to 'improve their immunity', a practice we do not approve. Furthermore, according to the AHS monitoring report of 2009 it seems unlikely that in the second half of 2009 a similar 'epidemic' of abortions has occurred in the Netherlands as in 2008.

Discussion

An epidemic of abortions in sows in the second trimester of gestation, like we assumed took place in 2008, apparently did not occur in 2009. In the investigated herds no evidence could be found for an infectious disease (PRRSv, PCV2, Chlamydophila or Coxiella) causing the abortions. Interestingly, all sows showed extremely high antibody titers against Influenza. Seroconversion against one or more types of PI was found in no more than 50% of the sows. In only one herd other symptoms like anorexia or fever that could indicate a PI infection, were seen. In 5 herds sows were vaccinated against PI. In conclusion, we still have to be alert on the next 'epidemic' of abortion in sows, meanwhile trying to improve diagnostic tools and controlling other non-infectious causes of abortions.

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CONVENTIONAL SOWS INSEMINATED WITH ARTIFICIALLY PCV2 INFECTED SEMEN: I. *IN VIVO* RESULTS

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Introduction

Since 1999, field evidence of transplacental infection by PCV2 and reproductive failure has been reported (1). Several experimental studies were performed: trans-uterine inoculation of foetuses (2), oronasal inoculation of pregnant SPF (3) or conventional (4) sows, intra-uterine inoculation of SPF sows (5). The objective of this study was to evaluate the clinical and pathological consequences of PCV2 infection in conventional pigs by artificially infected semen.

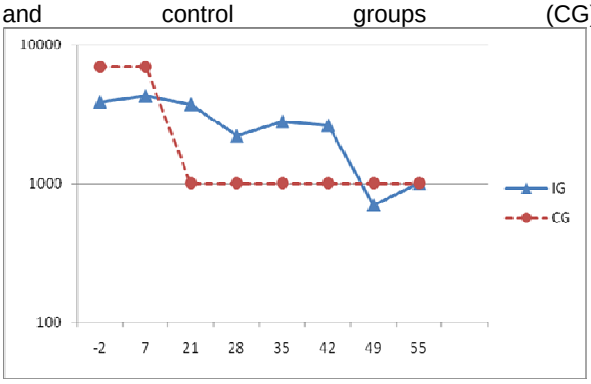
Material and Methods

Nine prepubertal conventional pigs were randomly divided: 3 controls and 6 infected. Hormonal oestrus synchronization was followed by artificial insemination (AI) with a single dose semen added with 10 ml of a PCV2 suspension. After ultrasonography at 29 day post-insemination (DPI), empty sows were euthanized at 30 DPI whilst pregnant ones between 52nd and 56th DPI. Cervix, nasal and rectal swabs, and blood samples were weekly collected from -2 DPI till the end of the experimental period. Serum samples were directly and indirectly tested for PCV2, PRRSV, PPV and ADV. Serum antibody titres were determined by testing serial dilutions of each serum, by competitive ELISAs (6, 7, 8). The protocol described by Olvera *et al.* (9) was employed for PCV2 real time-PCR. Serum progesterone levels were measured by radioimmunoassay (10).

Results

At the -2 DPI, none pig presented viremia for all tested virus. All animals showed a high anti-PCV2 antibody level at -2 DPI (range 1/1000-1/10000), but only one sow had a lower titre (1/100). Four out of 6 infected sows displayed viremia at 7, 21, 28 and 35 DPI (the subject with the lowest anti-PCV2 level kept showing positive blood results for two successive samplings at 21 and 35 DPI). This latter was also the only sow showing a positive swab (rectal, 35th DPI). The anti-PCV2 antibody proved a decline after 7 DPI followed by a plateau in controls, whereas in infected animals were recorded values higher than controls that declined only after 42 DPI (Figure 1). No sows showed any signs of oestrus after AI. Pregnancies were established in 3 out of the 6 infected sows and in 3 out of the 3 controls (one control sow aborted 3 foetuses at 21 DPI). Serum antibody titres against PPV, PRRSV and ADV (total and anti-gE) decreased between -2 DPI and at the last DPI. Progesterone was absent in all gilts at insemination and in all animals increased after oestrus induction but in the three non-pregnant infected animals never raised until the 28 DPI.

Figure 1: Mean anti-PCV2 antibody titre in infected (IG) and control groups (CG)



Discussion

In the present study the assessment of a field situation employing conventional pigs and intrauterine PCV2 exposition was investigated. Viremia was recorded in 4 out of 6 infected animals, besides mean antibody titre higher only in exposed subjects. Three infected sows out of 6 were not pregnant, unlike controls were all pregnant. Moreover, it should be emphasized that only the infected sow with lower PCV2 antibodies titre at -2 DPI showed simultaneously viremia and fecal virus spread. Therefore, i) the PCV2 infection is possible in conventional sows by intrauterine exposition; ii) low antibody titres increase the probability of the infection; iii) PCV2 infection close to insemination reduces the pregnancy rate.

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CONVENTIONAL SOWS INSEMINATED WITH ARTIFICIALLY PCV2-INFECTED SEMEN: II. *POST MORTEM* RESULTS

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Introduction

Experimental infections, using several routes of virus inoculation in pregnant animals brought at parturition (1,2,3,4), demonstrate the role of the PCV2 on reproductive pathology. It is known that *in utero* death of fetuses producing dead or mummified piglets at delivery infected with PCV2 can be assessed in tissues (1,3,5). In PCV2 associated reproductive pathology the presence of uterine or placental lesions are scantly documented. We developed an original experimental model of PCV2 infection by artificial insemination of conventional sows with PCV2 infected semen and in this paper we present and discuss the PCV2 tissue distribution and the associated lesions of the sow and fetuses after *post-mortem* samplings.

Material and Methods

The experimental design and the results of the *in vivo* investigation are contextually presented (6). Necropsy was performed on all the non-pregnant (n=3) and pregnant (n=6) animals. Several tissues of the sows and of each foetus (heart, lung, liver, placenta and amniotic fluid) were collected (Table 1) for real time-PCR, histology and immunohistochemistry (IHC). The corresponding uterine tract and the placenta were collected together with foetus sampling. Real time-PCR analyses were in accordance to the method by Olvera *et al*. (7). Samples for histology were stained with haematoxylin and eosin (H-E). IHC was performed using 1/100 PBS-diluted PCV2 antibody (Mab F217, by Dr. G. Allan (Belfast, UK)), as previously described (8) but using a streptavidin-biotin-peroxidase polymeric complex (SuperPicture kit peroxidise, Zymed® Lab).

Results

In the sows, at necropsy, the only lesions recorded included mild to moderate chronic fibrous pleuritis and/or pericarditis. One animal showed enzootic pneumonia. One case of a mild sero-fibrinous peritonitis and two cases of white spotted liver were observed. Two out of the 3 non-pregnant sows showed congestion and edema of uterine mucosa. Three out of 6 infected sows were pregnant and had respectively 16, 11 and 7 fetuses, whereas two out of the 3 controls 11 and 8. One control sow aborted but any PCV2-DNA was evidenced in the 3 aborted fetuses. In only one placenta (positive for PCV2 DNA with moderately high viral load, $>10^8$ genome copies/ml) mild focal necrosis of the chorionic epithelium resulted positive by IHC for PCV2 antigen localized in the cytoplasm of the cells. The foetus showed also IHC positive reaction in the cytoplasm of hepatocytes.

Table 1: RT-PCR results of tissue sampling

	Infected						Control		
	pregnant			not pregnant			pregnant		
sow ID	25	26	29	27	32	33	28	30	31
Number of foetuses	16	11	7	0	0	0	11	0*	8
Tonsil	-	-	-	+	-	-	-	-	-
Uterine left lymph node	+	-	-	-	-	-	-	-	-
Uterine right lymph node	+	-	-	+	-	-	-	-	-
Tracheo-bronchial lymph node	+	-	-	/	-	/	-	-	-
Cervix	-	-	-	-	+	-	-	-	-
Foetuses (pos/tot.)	10/16	-	3/7	-	-	-	-	-	-
Amniotic fluids (pos/tot.)	-	1/11	-	-	-	-	-	-	-
Foetus and corresponding uterine tract (left)	7	-	-	-	-	+	-	-	-
Foetus and corresponding uterine tract (right)	1	-	-	-	-	-	-	-	-

* Aborted 3 fetuses before necropsy

Discussion

The present investigation suggests the protective role of the anti-PCV2 antibodies in preventing foetal infection even in case of intrauterine route of infection. Sow n. 25, with the lowest anti-PCV2 titre (beginning of the experiment) (6) and viremia for two successive samplings, had also the highest number of positive fetuses and concordance with corresponding uterine tract positivity. Moreover, the results seem to demonstrate that PCV2 replicates also in chorionic epithelium where it was the possible cause of necrosis. As well as viral load in foetal tissues, also placenta lesions can account for foetus death. This study evidenced that not all foetuses are positive for PCV2, it should be recommended to send the whole litter to the lab for a correct diagnosis of PCV2-related reproductive failures.

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HEMATOLOGICAL PARAMETERS AND SERUM PROTEINS IN PIGS WITH PORCINE CIRCOVIRUS TYPE 2-ASSOCIATED DISEASE (PCVAD)

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Introduction

Clinical features of infection with Porcine Circovirus Type 2 (PCV2) include reproductive disorders and disease complexes such as Porcine Multisystemic Wasting Syndrome (PMWS), Porcine Dermatitis and Nephropathy Syndrome (PDNS) and Porcine Respiratory Disease Complex (PRDC) in weaned pigs (Segalés *et al.*, 2004). The characteristic depletion of lymphocytes and infiltration of macrophages and multinucleated giant cells in lymph nodes in pigs with PCV2-associated disease (PCVAD) lead to the hypothesis that pigs with PCVAD have altered hematological parameters and serum proteins compared with clinically healthy pigs.

Material & Methods

About 50 weaned pigs from eight herds with apparent PCVAD were sampled. Twenty-four pigs were confirmed to have PCVAD through detection of PCV2 in association with characteristic histopathological findings were subsequently included in the study. One hundred and four clinically healthy pigs at the same age were used as controls (Klem *et al.*, 2009). Blood samples added ethylenediaminetetraacetic acid (EDTA) were assayed using an ADVIA 2120 haematology system in the MultiSpecies™ System Software, while the proteins fractions in serum were determined by electrophoresis (Capillarys™ 2 Sebia). Statistical analysis was carried out using JMP Statistical Discovery Software Version 7.0.

Results

Pigs with PCVAD had significantly ($P<0.05$) lower numbers of erythrocytes, eosinophils, lymphocytes, monocytes and a lower hematocrit than healthy pigs. In addition, they had a significantly ($P<0.05$) higher number of neutrophils. Pigs with PCVAD had a significantly lower level of albumin than healthy pigs, but there is no significant difference in the total amount of globulin. However, pigs with PCVAD had a significantly higher level of α-globulin and β-1-globulin and a significantly lower level of β-2-globulin than healthy pigs, while there is no significant difference in the level of γ-globulin.

Hematological parameters (mean±SEM) and serum proteins in pigs with Porcine Circovirus Type-2 Associated Disease and clinically healthy pigs

Parameter	Healthy pigs	PCVAD
Erythrocytes ($x10^{12}$ #/l)	7.3±0.1	6.9±0.2
Hematocrit (l/l)	0.39±0.003	0.33±0.012
Leucocytes ($x10^{9}$ #/l)	27.8 ±0.8	24.2±2.8
Neutrophils ($x10^{9}$ #/l)	10.2±0.4	16.4±2.3
Eosinophils ($x10^{9}$ #/l)	0.71 ±0.07	0.32±0.05
Lymphocytes ($x10^{9}$ #/l)	14.4±0.6	5.4±0.9
Monocytes ($x10^{9}$ #/l)	1.8 ±0.1	1.3±0.2
Total proteins (g/l)	58.2±0.4	54.0±2.8
Albumin (g/l)	24.3±0.2	18.6±0.8
Globulin (g/l)	34.0±0.4	35.4±2.5
α-1-globulin (g/l)	4.3±0.1	5.5±0.3
α-2-globulin (g/l)	7.3±0.1	8.3±0.4
β-1-globulin (g/l)	4.5±0.1	6.1±0.4
β-2-globulin (g/l)	6.7±0.1	5.1±0.3
γ-globulin (g/l)	11.1±0.3	10.4±2.4

#, number; l, litre; g, gram

Discussion & Conclusions

The results confirm that pigs with PCVAD have altered hematological parameters and serum proteins compared with clinically healthy pigs. We think that these findings are important for understanding the susceptibility for other infections and the response to vaccination.

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COMPARISON OF THREE DIFFERENT SEROLOGICAL METHODS FOR THE DETECTION OF ANTIBODIES AGAINST PCV2

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Introduction

Over the last years, vaccines against PCV2 have been successfully used to control PCVAD and associated economical losses (1). However, it is still impossible to evaluate the efficacy of the vaccination by routinely used diagnostic methods (2). The aim of the present study was to compare three different serological methods with regard to their ability to detect or quantify antibodies in piglets vaccinated against PCV2 at different ages.

Material & Methods

The study was conducted in a 65 sow farrow-to-finish farm. In total, 126 piglets were randomly allocated to two different groups at 14 days of age. Group A received a single intramuscular dose of 1 ml Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica, Ingelheim, Germany) at 14 days of age. The same vaccine was administered to piglets of group B on the 28th day of life. Blood samples were collected in weeks 2, 4, 6, 8, 12, 16, 20, and 23 (A: n=10, B: n=10). All sera were analysed by the Ingezim Circovirus IgG/IgM (Ingenasa, Spain, data only shown for IgG) and the Serelisa® PCV2 Ab Mono Blocking (Synbiotics Europe SAS, France). Immunofluorescence (IFT; BioScreen GmbH, Muenster, Germany) was additionally conducted on samples of weeks 2, 8 and 23.

Results

All samples tested with Serelisa® or IFT remained positive throughout the trial, whereas the percentage of animals positive in the IgG ELISA decreased over time (Fig. 1). A decrease in antibody titers was observed in both quantitative tests between 2 and 8 weeks of age (data not shown). No significant difference in antibody titres was observed between the two treatment groups. Antibody levels measured by Serelisa® and IFT showed a highly significant correlation using Spearmans coefficient of correlation ($p<0.001$, $\rho=0.735$) over the whole period of the investigation. However, results for individual samples can differ (Fig. 2). The differences between the two tests were more evident at the end of the trial (week 23). Between 16 and 20 weeks of age 3/10 pigs vaccinated at day 14 and 2/10 pigs vaccinated at day 28 showed an increase in antibodies measured by the Serelisa®. Serum samples of those animals were tested by qPCR for the presence of PCV2 at 12, 16 and 20 weeks of age to exclude an infection. In none of the samples PCV2 antigen could be detected.

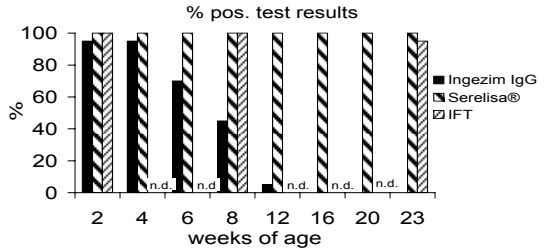

Fig. 1. Percentage of animals tested positive by the Ingezimtest, Serelisa® and IFT

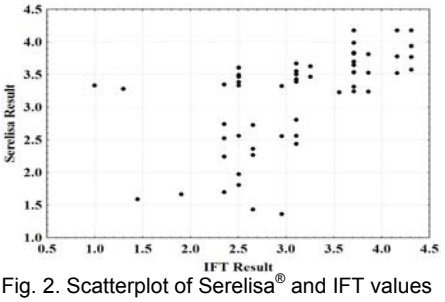

Fig. 2. Scatterplot of Serelisa® and IFT values

Discussion & Conclusions

In this study the Ingezim test seemed to be less sensitive to detect antibodies against PCV2 than the Serelisa® test or IFT. However, it should be kept in mind that it is not well defined what type of antibodies are measured with the different test systems. In general, the quantitative tests showed a good agreement. In individual samples differences of more than 2 log could be detected between the two tests. In addition, an increase in antibody titres in individual samples measured by Serelisa® could not be related to positive findings in the qPCR. Based on these findings quantitative results should be interpreted with care.

No differences in antibody titres could be detected between early and late vaccination. In both cases vaccination did not induce an increase in antibody titers. This is in line with other studies, where PCV2 vaccination did not induce seroconversion, but protected very well against PCVD (1). Therefore, serological analysis is not suitable to evaluate the efficacy of vaccination against PCV2.

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ANALYSIS OF SEROCONVERSION AFTER VACCINATON WITH DIFFERENT PCV2 VACCINES

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Introduction

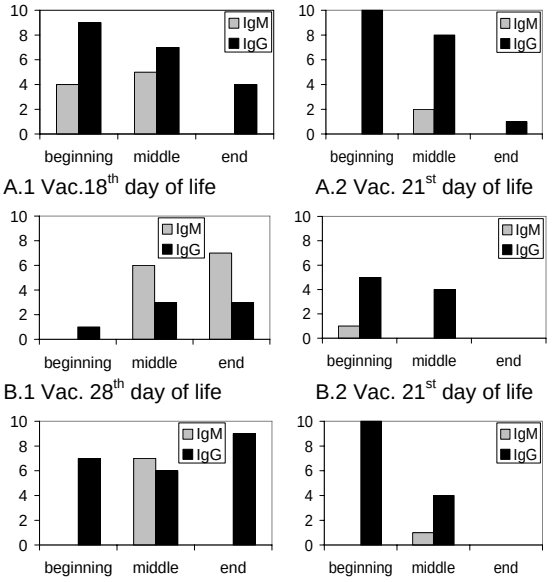
After the introduction of vaccines, a general decrease of PCV2 associated diseases was observed over the last few years (1). However, the evaluation of the efficacy of these vaccines is still difficult (2). The serological response after the vaccination doesn't give any information about the provided protection, but perhaps it can indicate that the vaccination was performed correctly. The objective of this study was to evaluate the serological response of piglets vaccinated with different PCV2 vaccines.

Material & Methods

The seroconversion of piglets after vaccination with three different PCV2 vaccines has been investigated. Each vaccine (A, B, C) has been administered to 10 animals in two different farms. Sows have not been vaccinated against PCV2 in any of the six farms. Vaccine A was administered on the 18th day of life in one farm (A.1) and on the 21st day of life in the second farm (A.2). B was given on day 28 (B.1) or 21 (B.2), while C was applied on the 28th (C.1) and on the 26th (C.2) day of life respectively (figure 1). Blood samples were taken from all pigs at the beginning, in the middle and at the end of the growing period. All sera were tested by Ingezim Circovirus IgG/IgM (pos/neg., Ingenasa, Madrid, Spain).

Results

The number of pigs positive for IgM and IgG at the beginning, in the middle and at the end of the growing period is presented in figure 1. A wide difference in seroconversion was detected concerning both, IgM and IgG production. Pigs vaccinated with vaccine A showed IgM production at the beginning and in the middle of the growing period in one farm. In the second farm pigs that were vaccinated with the same vaccine showed hardly any IgM production. Antibodies of the IgG class declined in both farms over the growing period. Vaccine B induced an IgM response in the middle and at the end of the growing period in one farm that was completely absent in the second farm. Pigs vaccinated with vaccine C showed IgM production in the middle of the growing period in both farms but the number of IgM positive pigs differed seriously. The highest number of IgG positive pigs was found at the end of the growing period in one farm. At the same time no IgG positive pigs were found in the second farm while all pigs of this farm were IgG positive at the beginning of the trial.



A.1 Vac.18th day of life A.2 Vac. 21st day of life

B.1 Vac. 28th day of life B.2 Vac. 21st day of life

C.1 Vac. 28th day of life C.2 Vac. 26th day of life

Figure 1: Number of pigs positive for IgM and IgG

Discussion & Conclusions

All results must be interpreted with care because the vaccines were used in different farms at different time points. In this study all three vaccines (A, B, C) could evoke a detectable serological response. Differences in antibody production were not only seen between pigs that were vaccinated with different vaccines but also between pigs of different farms that were vaccinated with the same vaccine. In general, it can be stated that all vaccines could cause an IgM reaction detectable by the test system. In unvaccinated piglets conclusions about the time point of infection (acute, subacute, chronical) can be drawn by detecting IgM and IgG. In the field it is not possible to determine if the onset of IgMs is due to the vaccination or caused by an infection with a field strain. As the results demonstrate, not all pigs show seroconversion after vaccination. Therefore, serological analysis cannot be used to evaluate the efficacy of vaccination against PCV2 (1).

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COMBINED MYCOPLASMA AND PVC2 VACCINATION INCREASED FINISHER PERFORMANCE

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Introduction

In the pig production of 2010, vaccination of pigs against a variety of infections is used to keep a high and steady level of performance in finishers. The use of vaccines against PCV2-virus is widespread, and in herds vaccinating against PCV2, the usual *Mycoplasma hyopneumoniae* (M hyo) vaccination might seem unnecessary, because the pigs perform very well with PCV2 vaccination only. This side-by-side study evaluates the benefits of vaccinating against M hyo in a Swedish herd already vaccinating against PCV2-virus.

Material & Methods

The herd was a Swedish finishing herd receiving batches of up to 400 pigs with 5 weeks interval. Two adjacent batches of pigs were included in the study. Each of these batches were divided in 2 groups; One group vaccinated against M Hyo and PCV2 (Ingelvac MycoFLEX® together with Ingelvac CircoFLEX®) and another group vaccinated against PCV2 only (Ingelvac CircoFLEX®). Pigs were vaccinated at 3 weeks of age, weaned at 5.5 weeks of age and moved to the finishing barn at 10.5 weeks of age. Each group of pigs was tattooed with different delivery numbers before slaughter. The slaughter date, carcass weight, lean meat % and carcass price was recorded for each pig at the slaughterhouse, as was the % of pigs having lung lesions. Comparison of pigs vaccinated with 2 vaccines to pigs vaccinated against PCV2 only was done with Wald-Wolfowitz runs test for days to slaughter and lean meat % and with Students t-test for carcass weight and carcass price (significance level p=0.05).

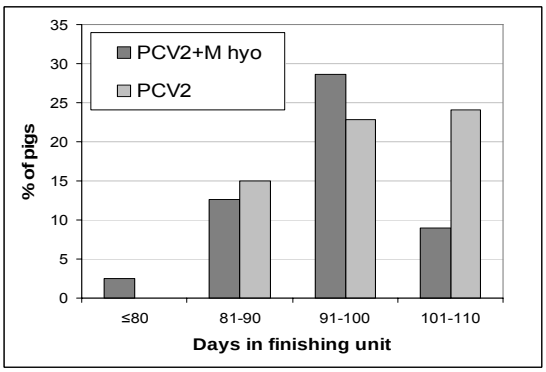

Fig. 1: Number of days in finishing unit

Table 1: Slaughterhouse data for performance.
**=Statistically significant difference between means*

Vaccination	PCV2 +M hyo	PCV2	Diff.
# of pigs slaughtered	436	319	
Birth to slaught (days)	173.8	174.9	**-1.1***
Carcase weight (kg)	87.0	87.2	**-0.2**
Lean meat (%)	57.46	57.27	**+0.19***
Price of carcase (€/pig)	114.3	112.9	**+1.4***

Results

The vaccination of pigs with both PCV2 and M Hyo vaccine resulted in a statistically significantly better performance of the finishers, compared to pigs vaccinated only against PCV2 (table 1). Double vaccinated pigs reached slaughter weight one day earlier than pigs vaccinated with only one vaccine, and the carcase quality was better, thus giving a higher price per pig. The increase in the price of the carcases was 2.3 times as high as the price of the M hyo vaccine. In fig. 1, the faster growth of pigs vaccinated with both vaccines is visualized.

Discussion & Conclusions

The present herd had no significant clinical problems with M hyo infection, and only 0-3% of the carcasses was getting remarks for pneumonic lesions at slaughter. Hence, the introduction of the M hyo vaccine was merely tested as a way to improve the overall performance of these finishers.

The study showed, that the performance was significantly improved by M hyo vaccination, increasing the mean price of the carcass by 1.4 € per pig. Therefore, vaccination against M hyo on top of the PCV2 vaccination gave a payback of at least 2.3 times the investment in the M hyo vaccine.

EFFECT OF VACCINATION OF AGAINST PCV2 AND LAWSONIA INTRACELLULARIS IN SPAIN: A FARM CASE

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Introduction

PCV2 and *Lawsonia intracellularis* are two of the most widely spread pathogens in Spain. Both are supposed to be endemic in all pig producing countries (1,2). The efficacy of both Ileitis vaccination (3,4) and PCV2 vaccination (5-7) has been demonstrated separately. The objective of this study was to evaluate a possible additive effect of PCV2 and Ileitis vaccination.

Material & Methods

The study was conducted in 12 farms of one cooperative with a maximum of 300 sows. The farms work in 3- or 4-week rhythm and piglets are weaned between 21 and 26 days. Sow unit and nursery are on one site. Finishing sites are located in a highly pig dense area and house animals placed at about 20 kg bodyweight originating from the different sow farms.

Sows of all farms were positive for PRRSV, APP and *Lawsonia intracellularis*.

This before-after study included 14,333 animals divided in 4 groups: 1 - Non vaccinated, 2 - Vaccinated against Ileitis (Enterisol® Ileitis, 2ml via drench few days before weaning), 3 - Vaccinated against PCV2 (Ingelvac CircoFLEX®, 1ml i.m. at weaning, 3-4 weeks of age) and 4 - Vaccinated against Ileitis+PCV2 (same administration as groups 2 and 3). All animals were kept under similar husbandry conditions. All sow farms had the same sow vaccination protocol and all piglets were vaccinated during 1st week of life against *Mycoplasma hyopneumoniae*. During the whole study the same nutritional scheme and in-feed medications were followed for sows, piglets and pigs.. Performance of all treatment groups was compared by SPC (Statistical Process Control), using Statistica® Version 8.0, Statsoft.

Results

Overall the performance data improved in the vaccinated animals compared to the non-vaccinated animals and the medication costs were drastically reduced (table 1). The mortality rate was statistically different between each treatment group (figure 1).

Discussion & Conclusions

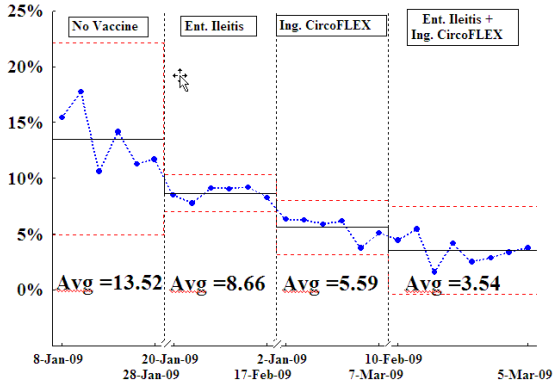
In the present study the animals vaccinated against Ileitis or PCV2 performed better than the non-vaccinated pigs.

Table 1. Relevant parameters in the 4 different groups of vaccination.

	No Vac	Ileitis	PCV2	Ileitis+PCV2
Num piglets	2614	3102	3888	4729
Culls (%)	11.7 [a]	10.2 [a]	3.4 [b]	2.4 [b]
Days of fatt.	158.7	142.5	140.2	141.4
ADG (g/day)	604	672	659	667
FCR (kg/kg)	3.69	3.15	3.31	3.08
Medication costs (€/pig)	3.72	2.81	2.23	1.85

a,b: values with a different superscript, differ significantly ($p<0.001$).

Figure 1. SPC chart of Mortality (%)



Additionally an additive effect on all measured parameters could be observed in the animals vaccinated against PCV2 and Ileitis. Also medication cost could be halved through Ileitis plus PCV2 vaccination.

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ECONOMIC RETURN FOLLOWING VACCINATION WITH INGELVAC CIRCOFLEX® IN A FARM WITH MODERATE PCVD PRODUCING HEAVY PIGS

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Objective

Porcine Circovirus Diseases (PCVD) represent one of the biggest challenge for the swine industry worldwide. In 2008 Ingelvac CircoFLEX® was the first PCV2 piglet vaccine that became available in the EU and positive impact on economic return in acute PCVD affected farm by using CircoFlex was demonstrated (1). Italy has a very long production cycle, producing a unique heavy pig slaughtered at minimum 270 days of age: the duration of immunity up to 9 month after vaccination, has been also already described (2). The objective of this study was to evaluate the economic benefit following vaccination with Ingelvac CircoFLEX® in a farm presenting moderate PCVD and producing heavy pigs.

Materials and methods

The study was carried out in a multisite system, comprising a total of 2000 sows. Piglets were moved to site 2 at about 10 weeks of age and to site 3 at 20 weeks. The animals suffered from moderate form of PCVD, with total losses from about 10 weeks of age to slaughter averaging 12 %. Data coming from 12 consecutive groups were collected: six unvaccinated groups (n=7199 pigs) and six vaccinated (n=7367 pigs). Vaccinated animals received a single dose (1 ml) of Ingelvac CircoFLEX® around weaning day (28 days of age).
Mortality and runts were recorded per group, from transfer in site 2 and site 3. Runts were defined as those pigs sold as underweighted pigs or culled because of severe disease.
Mortality and runts were compared using a Chi-square test (Statitistica® v8.0, Statsoft®, USA).
In terms of economics, opportunity costs for the mortality and runts were considered. In principle it is the value of the next-best choice available to someone who has picked between several mutually exclusive choices. In this study, the sales value of an average marketed pig is considered. Variable costs saved by a pig not reaching market (e.g. feed) are substracted. Economic assumptions taken were: feed price of €200/ton, FCR of 3,9, market price for heavy pig of €1,25/kg live weight, runt value of €0,3/kg live weight.

Results

A total of 6303 controls and 6776 vaccinated pigs were slaughtered at 42 weeks of age. Slaughter weeks ranged for control groups from the 1st March till 4th April 2009, followed by vaccinated from 1st May to 2nd June 2009. Week 10 to slaughter mortality and runts were significantly reduced in vaccinated pigs compared to controls. Total losses in the control group reached 12,4% while in the vaccinated it stopped at 8,0% ($p<0,0001$).

Table 1. Mortality and runts summary
P-values ≤ 0.05 indicate a statistical significant difference, p-value > 0.05 not significant (n.s.).

Period	Parameter	Circo-FLEX	Control	p-value
Site 2	Mortality, %	1,4	1,8	ns
	Runts, %	1,2	2,4	< 0,0001
	Total losses, %	2,6	4,2	< 0,0001
Site 3	Mortality, %	2,2	4,2	< 0,0001
	Runts, %	3,3	4,4	0,0007
	Total losses, %	5,6	8,6	< 0,0001
Total	Mortality, %	3,6	5,8	< 0,0001
	Runts, %	4,5	6,6	< 0,0001
	Total losses, %	8,0	12.4	< 0,0001

An opportunity gain of €5,45/head was calculated in favour of the Circoflex vaccinated pigs.

Discussion and conclusion

The present field study demonstrates that Ingelvac CircoFLEX® provides significant benefits in a multisite production system, suffering from a moderate form of PCVD, where pigs are slaughtered at 42 weeks of age. The economic differential from the implementation of vaccination was calculated based on the reduction in total losses in site 2 and 3 (mortality + runts). The significant reduction in mortality and runts lead to a clear economic advantage of €5.45/head in the vaccinated group.

References

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(2) Terreni et al. (2009) ECPHM proc.: 74.

FULL DOSE VS HALF DOSE OF INGELVAC CIRCOFLEX: FIELD DATA IN SPAIN

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Introduction

Every day more, PCVD vaccination is becoming a routine in pig production, in some countries, vaccination rates are reaching extremely high percentages, like in US, about 95% in early 2010. Efficacy and return of investment have been demonstrated in several cases of PCV2 vaccination (1, 2).

On the other hand, since 2006, huge economical and financial crisis is affecting pig producers all over the world. Reducing input costs is one of the main objectives, therefore some producers in Spain decided to cut doses of PCV2 vaccines.

The objective of this study is to compare the efficacy of a PCV2 vaccine, Ingelvac CircoFLEX® (Boehringer Ingelheim) at full dose in comparison to half dose in mortality, medication costs and fattening performance.

Materials and Methods

This study was performed in a farrowing farm with 750 sows. Herd is PRRS positive, sows are vaccinated against Aujeszky's disease, Parvovirus and porcine Erisypelas. Gilts come from external source and before entering the farm, are vaccinated against Aujeszky's, Parvovirus, Erisypelas and PRRS.

Piglets are weaned between 21 and 24 days and delivered to 3 nursery farms. All piglets are vaccinated against Mycoplasma at 7days of age and against PCV2 at weaning.

Piglets remain in the nursery farm until they reach 20kg of live weight, when they are moved to fattening units. In fattening, animals suffered moderate problems of PCVD. Peak of clinical signs were observed between 16 and 17 weeks of age. Before introduction of PCV2 vaccination mortality in fattening averaged at about 6% with medication costs of more than 3€ per pig, animals did not react to antibiotic treatments for secondary infections.

A total of 4.250 animals were included in this before-after study. First, 3 fattening batches (1.700 animals) were vaccinated at half dose (0,5ml per piglet) of Ingelvac CircoFLEX® off label. After that, 4 batches (2.550 animals) were vaccinated at full dose (1ml per piglet) following label recommendations.

All data presented is from fattening units. Mortality in both groups were evaluated using a Chi-square test.

Results

Animals vaccinated with full dose perform better than animals with half dose, (table 1). Weight gained in the fattening unit was 1,8 kg more in the vaccinated group with full dose spending 2 days more in fattening.

Difference in mortality was highly significant ($p<0,001$), obtaining a reduction of 56% in the group vaccinated with full dose in comparison with the results with half dose. Medication costs were reduced by 41% in the group of full dose.

Table 1. Relevant parameters

	Half dose	Full dose	Diff
# piglets tested	1700	2550	
Mortality (%)	4,8[a]	2,1[b]	-2,7
Weight gain (kg)	94,1	95,9	+1,8
Medication costs (€/pig)	2,59	1,53	-1,06

a,b: values with a different superscript, differ significantly ($p<0,001$).

Discussion & Conclusions

When comparing data of non vaccinated animals with animals vaccinated at half dose, some improvement was observed, but the clinical situation and mortality was still not satisfactory. Data presented in this field study, demonstrate that using full dose of Ingelvac CircoFLEX® instead of half dose improved the performance and lead to a significant reduction in mortality.

Based on reduction in mortality and medication costs, a yearly return of investment of 5,07:1 for every extra € spent on vaccine was calculated when moving from half to full dose of Ingelvac CircoFLEX.

The results of this study show that using full dose of Ingelvac CircoFLEX (compared to half dose) is improving the health situation on the farm and provides significant economic benefits.

References

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VACCINATION WITH INGELVAC ® CIRCOFLEX ™ UPON PLACEMENT IN THE FINISHING UNIT. A CASE REPORT FROM SWITZERLAND.

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Introduction

Infections with the porcine circovirus type 2 (PCV2) have enormous impact on health and performance of fattening pigs. Vaccination of piglets around weaning is the most effective tool to control diseases related to PCV2 infection. However, in early 2009 it was not always possible to buy PCV2 vaccinated weaners in Switzerland, as only a limited number of breeders used to vaccinate suckling piglets. Therefore, effect of vaccination against PCV2 upon entering the fattening farm should be studied.

Material & Methods

In a finishing farm with continuous pig flow and 220 places losses were incurred due to PCV2-related diseases.

100 pigs from 3 origins (A, B, C) were vaccinated (1ml Ingelvac ® CircoFLEX ™ im) upon entering the finishing farm with ø 26.3 kg. The effect of vaccination was followed by clinical observations and pathological analyses. Blood samples were collected from 30 pigs at vaccination time. Ten of these 30 animals were sampled 2 and 4 weeks later, and 4 of the 10 pigs a fourth time at week 10. Serum samples were analysed for presence of PCV2-antigen by quantitative PCR. Slaughter data were compared to data from previous, unvaccinated fattening groups.

Results

No adverse reactions to vaccination were observed.

Three pigs wasted and were dissected after euthanasia. One pig died and was dissected as well. Pathological examination revealed endocarditis valvularis (2x), gastric ulcers (2x) and proliferative ileitis due to *Lawsonia intracellularis* infection (1x). No PCV2-typical lesions in the lymphatic tissue were observed in histology and only sporadic IHC positive macrophages occurred. In the quantitative PCR, some pigs were positive at time 0 and stayed positive, pigs from origin C were negative at all sampling times.

The farmer continued with the vaccination at delivery. So, 3 unvaccinated groups could be compared to the first and the 2 following vaccinated fattening groups. Losses decreased after vaccination from ø 7.2% in the 3 unvaccinated groups to ø 2% in the 3 vaccinated batches. The average fattening period was reduced by 5 days (unvaccinated: 118.7 days, vaccinated: 113.5 days). Average daily weight gains are shown in table 1.

Tab. 1: Daily weight gain in g/d in 3 vaccinated and 3 non-vaccinated fattening groups and average values.

	Non-vaccinated	**Vaccinated**
winter	674	758
spring	763	815
summer	693	732
Average	**710**	**768 (+58)**

Discussion & Conclusions

Blood analysis showed, that PCV2-positive and PCV2–negative pigs were vaccinated. Both tolerated the vaccine well. The vaccine does not eliminate the virus, but supports natural defence and thus protects against PCV2-disease[1]. Pathological results indicate management problems or other diseases as ileitis. PCV2-associated diseases were not found. Mortality and days to slaughter were reduced, as well as average daily gain increased in vaccinated batches compared to non-vaccinated pigs. This indicates that PCV2 vaccination at entering the fattening farm was still useful, despite the fact that some pigs were already PCV2 positive. However, vaccination of infected pigs is a compromise and field experience as well as various trials have shown that pigs in the nursery can already benefit from PCV2 vaccination[2].

Nowadays, in Switzerland about 80% of the piglets are vaccinated against PCV2 around weaning.

References

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FIELD TRIAL COMPARING TWO PCV2 VACCINES ON A FARM WITH LATE PCV2 INFECTION

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Introduction

This study took place on a farrow-to-finish farm of 700 sows in France. In Q3 2008, the mortality rate in fattening reached 5.8% and average daily gain (ADG) over the same period 794 g/d. Circovac® vaccination was routinely implemented in sows (2ml) since 2006 and in piglets of four weeks of age (0.5ml) since Sept. 2007. A screening of the farm in Sept.-Oct. 2008 demonstrated the presence of PMWS cases at the end of fattening. From that time point, the veterinarian in agreement with the farmer decided to change the routine PCV2 vaccination in piglets to Ingelvac CircoFLEX®. Two months later, a study aiming at comparing the ability of both vaccines in preventing the impact of the PCV2 infection on growth performances and mortality was implemented.

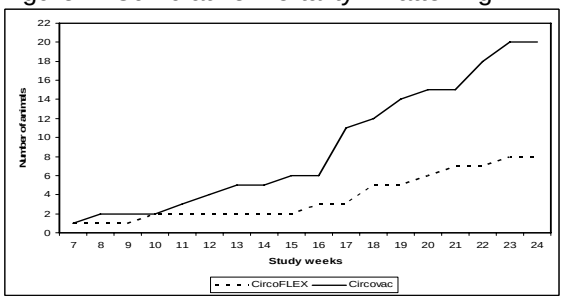
Material & Methods

A total of 1185 4-week-old piglets originating from Circovac® vaccinated sows were included over three week groups (WG1, WG2 and WG3) and divided into two treatment groups: CircoFLEX group (n=535) injected with Ingelvac CircoFLEX® (1ml IM) and Circovac group (n=548) injected with Circovac® (0.5ml IM). A control group (n=102) injected with water (1ml IM) was included to demonstrate presence of PCVD during the trial. To ensure the same housing and infection conditions, all the study animals were co-mingled throughout the study. Approximately 8% of the control animals were sampled at 5 time points (4, 10, 15, 19 and 22 weeks of age) to identify the time and profile of the PCV2 infection by Real-Time PCR. All study animals were weighed three times (4, 11 and 23 weeks of age).

Results

PCV2 infection was confirmed to occur after 15 weeks of age (study week 11) with a peak at the end of fattening. The presence of PCV2 was further confirmed in necropsies (including IHC). During the fattening period (11 to 23 weeks of age) when the PCV2 field infection took place, the mortality was significantly reduced in the CircoFLEX® group (1.5%) when compared to the Circovac® group (3.7%, p=0.0338). The increase in mortality coincided with the onset of viremia (Figure 1). Over the same period, the CircoFLEX® and Circovac® groups out-performed the control group respectively by 1.75 kg (p=0.0640) and 1.46 kg (p=0.1226). Weight gain between the two treatment groups did not differ significantly (p=0.5881). When interpreting the differences between the two treatment groups and the control group, it should be taken into account that the control group was primarily included for epidemiological reasons and only comprised 102 pigs.

Figure 1: Cumulative mortality in fattening.



Discussion & Conclusions

The presence of PCVD on the farm and the late onset of PCV2 infection were confirmed in control animals during the study, demonstrating that sow vaccination does not protect pigs through to slaughter. In addition, Ingelvac CircoFLEX® provided superior protection compared to Circovac® used in piglets, as shown by a significant reduction in mortality. A significant difference in weight gain between the two treatment groups was not expected as the PCV2 infection occurred late. Furthermore, it is likely that the higher mortality rate in the Circovac® group positively influenced the weight gain by eliminating the poor doers from the calculation. Beside the study results, the superior efficacy of Ingelvac CircoFLEX® is confirmed by the consistently good performance after implementing piglet vaccination with Ingelvac CircoFLEX®. Indeed, in Q2 and Q3 2009 when all pigs where vaccinated with Ingelvac CircoFLEX® mortality in fattening was 3.1 and 3.8%, compared to 4.9 and 5.8% in Q2 and Q3 2008 when only Circovac® vaccinated pigs where present, reflecting a reduction in mortality of about 2%, very similar to what has been observed in this side-by-side study.

IFN-γ SECRETING CELLS RESPONSE AFTER VACCINATION WITH A SINGLE DOSE OF A PCV2 VACCINE

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Introduction

PCV2 vaccines have been demonstrated to protect against PCVD and to be cost-effective. It has been shown that cell-mediated immunity plays a role in induced protection [1]. The present study was aimed at investigating PCV2-specific cellular immune response, evaluated as the number of IFN-γ secreting cells after vaccination and their division in classes of responsiveness.

Materials & Methods

At inclusion (weaning - 21±3 days of age), 20 pigs received a 2 ml intramuscular dose of Porcilis PCV® (vaccinated group). As controls, 20 pigs (placebo/control group) were injected with 2 ml of adjuvant (Diluvac Forte®). All pigs were bled at day 0, and at 7, 14, 21, 28 and 42 days post-vaccination (PV). The levels of IFN-γ secreting cells (SCs) in PBMC were determined by an ELISpot assay according to Martelli et al. (2009) [2]. The *ex vivo* recall response was stimulated by the addition of a field PCV2 strain solution (whole virus I12/11) and tested at 0.05, 0.1 and 0.25 multiplicity of infection (MOI). Individual responses were classified, according to responsiveness, as no/poor (0-40), low (45-100), intermediate (105-200), high (205-400) and very high (>405) response. No/poor responsiveness was established based on the unspecific cellular response observed in un-vaccinated pigs (data not shown) and according to recent literature [3].

Results

A single dose of PCV2 vaccine elicited a strong cell-mediated immune response measured as IFN-γ SC. From 2 weeks PV, the frequency of PCV2-specific IFN-γ SCs increased markedly in the majority of pigs upon re-stimulation with all the MOI used. A statistically significant dose-dependent response was observed. From 2 to 6 weeks PV, high cellular reactivity was maintained and the mean levels ranged between 75 and 225 IFN-γ SC/10^6 PBMC. Results from the analysis of responsiveness showed an inter-individual variability of the cellular response. At three weeks PV, approximately 70% of the vaccinated pigs were IFN-γ PCV2 specific responders.

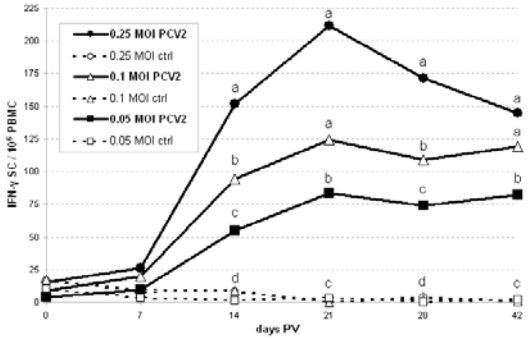

Fig. 1: Levels of PCV2-specific IFN-γ secreting cells (SC) in PBMC after single dose-vaccination. Courses detected upon different *ex vivo* whole field PCV2 re-stimulation (MOI).

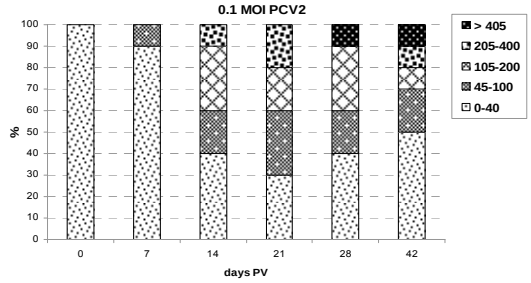

Fig. 2: PCV2-specific IFN-γ responsiveness categories (IFN-γ SC /10^6 PBMC) vaccinated pigs upon *ex vivo* re-stimulation with 0.1 MOI of whole field PCV2.

Discussion & Conclusions

After vaccination, an intense PCV2-specific IFN-γ SC response to a single dose of vaccine was detected and *ex vivo* IFN-γ secretion was not inhibited by incremental MOI with whole PCV2 virus re-stimulation, indicating effective antigen recognition and T cell activation and memorization. Furthermore, high and very high responders were detected after 3 weeks PV. A single dose of PCV2 Cap protein-based vaccine can elicit a strong cellular immune response in the majority of vaccinated pigs, despite the high individual variation which may depend on intrinsic host factors and on the type of the stimulus used for the *ex vivo* recall.

References

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PORCINE CIRCOVIRUS 2 AS A CAUSATIVE AGENT FOR SEVERE RESPIRATORY SIGNS AND POLYSEROSITIS ON A SPECIFIC PATHOGEN FREE FATTENING FARM

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Introduction

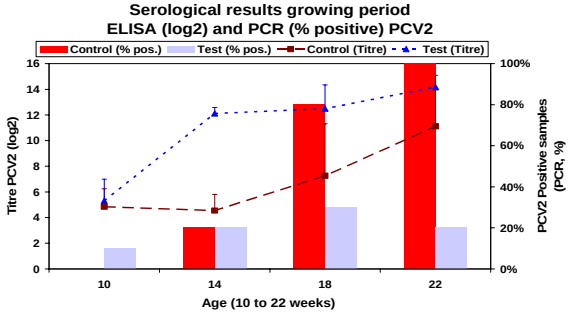
The clinical expression of PCVAD embraces a complex of signs including those related to the respiratory system.. Experimental PCV2 infections in Specific Pathogen Free (SPF) pigs has led to severe respiratory signs with high mortality [(1)]. In 2009 on 2 locations SPF fattening pigs from one origin suffered from severe respiratory symptoms (dyspnea, coughing), high fever and a high level of mortality in spite of vaccination at 4 and 6 weeks of age against *Haemophilus.parasuis* (Porcilis®Glässer). Necropsy revealed polyserositis with negative bacteriological results, hyperemia of the lung, and interstitial lung edema. Organs were highly positive for PCV2 by IHC. Paired serological investigation of acutely sick pigs, only revealed seroconversion to PCV2. This strongly suggests that the clinical signs were related to PCV 2 infections in a similar situation as described in experimentally infected SPF pigs by Gauger et al[(1)]. A PCV-vaccination trial was set up in order to help the farm and to explore the relationship between the signs observed and PCV2 infections. After this trial, all new piglets that entered the fattening units were vaccinated.

Material & Methods

In a batch of 350 piglets, 197 were vaccinated with a single dose of Porcilis® PCV at 10 weeks of age, on their arrival in the fattening unit. The rest were left unvaccinated. Vaccinated and unvaccinated pigs were housed site by site in three different units. Mortality was recorded and in each trial group serum of 10 pigs was sampled at 10-, 14-, 18- and 22-weeks of age. Sera were tested on antibodies against PCV2 (Synbiotics), PRRS(Idexx), *H. parasuis* (Biovet), *M. hyopneumoniae* (Idexx) and the 42 kD outer membrane protein of *A. pleuropneumoniae* [(2)]. Sera were also tested on PCV2 virusload by qPCR. Average daily intake (ADI), feed conversion rate (FCR) and average daily gain (ADG) during 2 months after the start of the vaccination was compared with the results of not vaccinated pigs during 2 months before the trial.

Results:

Mortality	n	mort. n	mort %	Techn (25-112 kg)	ADG g/d	FC kg/d	ADI kg/d
vacc	197	3	1,5	1-07-09 - 31-08-09	786	2.71	2.29
controle	153	10	6,5	1-09-09 - 31-10-09	868	2.67	2.37



All samples were negative against PRRS and *M. hyopneumoniae*. Also *A. pleuropneumoniae* titers stayed at low levels in both groups. Some sera of the 10- and 14-week old fatteners were positive against *H. parasuis* in both groups.

Discussion & Conclusions

The clinical signs (respiratory distress and polyserositis) on this farm were likely caused by PCV2, no other causative agent was found in the diseased pigs and vaccination against PCV was successful in preventing the disease. Pigs vaccinated with Porcilis® PCV had a reduced mortality, a clear humoral response to vaccination and the amount of PCV2 virus positive pigs was reduced. The historical data show a clear improvement in ADG, ADI and FCR after starting the vaccination. The respiratory signs in this clinical field trial with SPF pigs were similar to those of experimentally infected SPF pigs after PCV 2 challenge [(1)]. The authors are not aware of any reports relating polyserositis to PCV2 infection. A possible explanation for this feature could be a co-infection with *Mycoplasma hyorhinis* [(3, 4)], an opportunist which is capable of causing polyserositis. Its involvement may be triggered by the immune suppression caused by PCV 2 infection. The practical implication of the results of both this and the Gauger trial is that PCV 2 can be the cause of acute respiratory signs with high fever and high mortality levels.

References

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EFFICACY OF A SINGLE DOSE OF PORCILIS PCV APPLIED AT WEANING ON A DANISH HIGH HEALTH FARM WITH PMWS

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Introduction

Circovirus type 2 (PCV2) is one of the agents responsible for PMWS and PCVD, major causes of losses in pig production. Porcilis PCV has been developed to reduce the losses due to infection with PCV2. Vaccination of piglets with a single 2 ml dose at 3 weeks of age results in both humoral and cellular immunity (1). The purpose of this study was to investigate the efficacy of Porcilis PCV vaccination of piglets in a field case of naturally occurring PCVD.

Material & Methods

A Danish pig farm received 250 piglets every other week from the same sow herd. For the first 8 weeks, the 250 piglets were housed in a separate section containing nine pens, which had previously been cleaned, disinfected, dried and heated. At around 30 kg, the pigs were moved to finishing accommodation operating continuously.

The herd's general health was very high (Danish SPF-declaration: SPF +AP12 - meaning free from all serotypes of *A pleuropneumoniae* (except serotype 12), *M hyopneumoniae* and PRRS. Three months before the start of the trial, classical PMWS was diagnosed in piglets 4 weeks after introduction onto the farm. When the trial began, the clinical picture had changed, and the disease was classified as PCVD.

Out of a batch of piglets delivered to the farm, 100 pigs were vaccinated with 2 ml of Porcilis PCV on the day of arrival, and 100 pigs were left unvaccinated. These 200 pigs were randomly allocated to pens of 32-34 piglets each, thus mixing vaccinated and unvaccinated pigs in the same pen. This scheme was repeated with the next batch of piglets delivered two weeks later, in a separate section, thus providing, in all, 200 vaccinates and 200 unvaccinated controls.

For each batch, 3 vaccinated and 3 unvaccinated pigs from each pen were bled on days 0 (day of vaccination), 18, 56 and 109. The samples were analyzed by qPCR for PCV2 virus. On days 0, 56 and 109, all the pigs were weighed individually.

Statistical analysis of the ADG was by varians analysis (multifactorial ANOVA, Statgraphics software), and of mortality by the Chi-square test.

Results

During the trial, serious tail-biting occurred in one pen. Half of the deaths recorded were the result of euthanasia after tail-biting and lameness. Apart from this, most cases of lameness and meningitis were cured by appropriate antibiotic therapy. The blood samples showed that the pigs were viraemic from day 56 onwards, throughout the trial period. Vaccinated pigs had lower levels of virus than the unvaccinated controls (Data not shown).

Period	Porcilis PCV	Control	P-value
ADG 0-8 weeks (no of weaners)	**369 (195)**	**356 (192)**	**NS**
ADG 8-16 weeks (no of finishers)	**792 (186)**	**753 (183)**	**0.0091**
ADG 0-16 weeks (no of pigs)	**587 (186)**	**561 (183)**	**0.0143**
Mortality (all pigs)	**11/200**	**18/200**	**NS**

Table 1. Average Daily Gain (ADG) in grams for different growth periods and overall mortality.

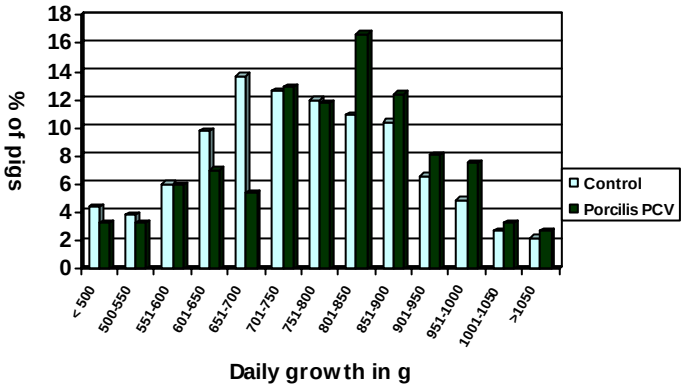

Figure 1. ADG of different growth classes.
The distribution shows a shift to the right for the pigs vaccinated with Porcilis PCV, indicating the better performance of the vaccinated pigs.

Discussion

The results showed that, even in a contaminated environment, with vaccinated pigs in direct contact with unvaccinated viraemic pigs, Porcilis PCV vaccination at weaning resulted in a significantly better ADG of 39 g during the finishing period. This is in accordance with the claim, in the SPC for Porcilis PCV2, that vaccination reduces weight loss due to infection with PCV2 (2). Since no other serious pathogens were present in the farm, the effect is related to vaccination against a 'pure' PCV2 infection.

References

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COMPARATIVE HUMORAL IMMUNITY RESPONSE TO VACCINATION WITH PORCILIS®PCV, A SECOND COMMERCIAL VACCINE AND UNVACCINATED ANIMALS

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Introduction

Monitoring the correct humoral immune response to vaccination is important when using routine vaccination procedures at early ages (3-4 weeks of age). This can be considered as the first step to assess possible interferences of vaccination with maternally-derived antibodies, and gives an indication about the response that will be obtained in subsequent production phases.

Material & Methods

The study was performed in a farm housing 1680 sows, with production facilities in three different sites, located in central Spain. Pigs from this farm showed evident PCVAD-related problems during fattening. The symptoms appeared at first at 13 weeks of life, and the most critical phase of the disease concluded 4 weeks later. Sixty-three animals with a mean age of 24 days (2 days before weaning) were selected for the study. The animals were uniquely identified using double ear-tags and were randomized into 3 groups, according to their dam and its parity, and piglet weight and gender, as follows:

- Porcilis®PCV group, single 2 ml dose, 21 piglets.
-Vaccine A group, single 1 ml dose, 232 piglets.
-Control group, single 2 ml dose of Diluvac Forte® (as a placebo), 19 piglets.

A commercial test was used for the detection of IgG and IgM (Ingezim PCV2 ELISA®, Ingenasa), comparing the results obtained at 3, 7 and 9 weeks of age. The cut-off thresholds differed for each antibody detection time: from the 1st to the 3rd sampling, the values for IgG were 0,7, 0.69 and 0.65, respectively and 1.02, 0.74 and 0.71 for IgM, respectively.

Statistical evaluation was performed using the Kruskall - Wallis test, the Pearson's chi-square test, the Fisher's exact test and the Mann-Whitney-U test.

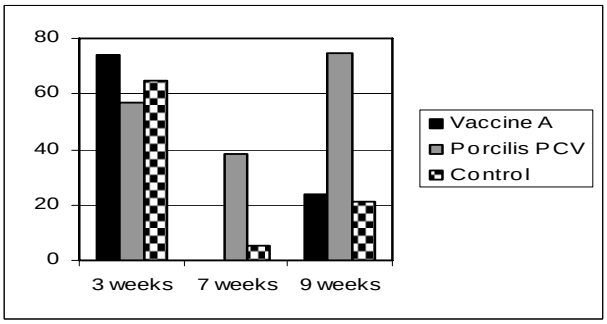
Results

The first sampling time coincided with the administration of the three treatments, and all the groups presented high levels of maternally-derived immunity, as shown by the high mean optical density ratio (OD) values and the high percentage of animals positive for IgG (Graph 1). Very low or negative values were found for IgM. All the parameters were comparable ($p<0.05$).

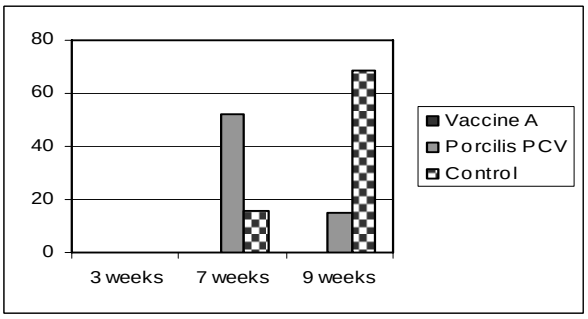
The second sampling at 7 weeks showed a stronger IgG response to vaccination in the Porcilis®PCV group, with significant differences for the percentage of positive animals compared to the other two groups ($p<0.05$). Vaccine A did not present significant differences vs. the Control group for both parameters ($p<0.05$). The Porcilis®PCV group showed a stronger IgM response (Graph 2), with significant differences when comparing the percentage of positives vs. the Control group ($p<0.05$) and vs. Vaccine A ($p<0.001$), with no differences between the Vaccine A and Control groups. The percentage of animals of the Vaccine A group positive for IgM at 4 weeks post vaccination was 0% (absence of serum antibody response).

At week 9, a marked increase of the percentage of positives vs. the other two groups was observed ($p<0.05$). No significant differences were found for both parameters between the other groups. A strong IgM response occurred in the Control group. Thus, when comparing the percentage of positives of the control vs. the vaccinated groups, significant differences were found vs. the Porcilis®PCV group ($p<0.05$) and the Vaccine A group ($p<0.001$). No significant differences occurred between both vaccinated groups ($p<0.05$).

Graph 1: % Samples positive for IgG



Graph 2: % Samples positive for IgM



Discussion & Conclusions

The strong response to the Porcilis® PCV vaccine obtained 4 weeks after vaccination, contrary to the findings for the Vaccine A and Control groups, indicates a correct immunisation of the animals, thus preventing interferences with maternally derived immunity.

Even though the farm had a history of late problems, the increase of IgM values in the Control group at week 7, which was more evident at week 9, probably was due to contact with the field virus.

SEROLOGY AND SAFETY OF THE SIMULTANEOUS USE OF PORCILIS® PCV AND M+PAC® IN THE FIELD

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Introduction

Porcine Circovirus Type 2 (PCV2) and *M.hyopneumoniae* vaccines are probably the most frequently used in the pig industry all over the world. As both vaccines are very often given at weaning, their simultaneous use would simplify herd management and improve animal welfare. The simultaneous administration of various commercial products has previously been shown to be effective (1). The object of this trial was to demonstrate that the simultaneous administration of Porcilis® PCV and M+PAC® is safe and efficacious in terms of serological response.

Material & Methods

The trial was performed on a 250-sow farrow-to-finish herd in north-east Spain, in which piglets had been routinely vaccinated with another PCV2 vaccine at 4 weeks of age, but had not been vaccinated against *M.hyopneumoniae.*

Porcilis® PCV is a subunit vaccine containing the viral capsular protein coded by the ORF2 of the PCV2 genome adjuvanted in X-Solve®. M+PAC® is an inactivated *M.hyopneumoniae* vaccine in Emunade®, an oil-in-water dual-action adjuvant.

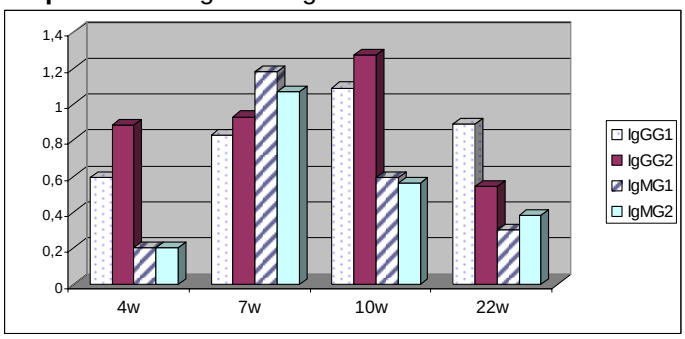
A total of 397 four-week old piglets were allocated to two experimental groups:
Group 1: 197 piglets, vaccinated with a mixture of 2ml Porcilis®PCV and 2ml M+PAC® injected in a single site on the left side of the neck.
Group 2: 200 piglets were vaccinated with 2ml Porcilis® PCV and 2ml M+PAC® in separate sites on either side of the neck.
All animals were identified individually by ear tag, and 10 animals of each group were bled at 4, 7 and 10 weeks of age.
All the pigs were monitored for signs of local or systemic reactions.
The immune response to Porcilis® PCV was evaluated comparing the PCV2 IgG and IgM titers of each group using Ingezim® PCV-ELISA (Ingenasa, Madrid, Spain).
Levene test was used for the comparison of variances and Mann-Whitney U-test for the comparison of means.

Results

No local or systemic reactions were observed in any of the animals of Group 1 (mixed vaccination). One piglet of Group 2 (separate vaccinations) exhibited a transient systemic reaction which rapidly disappeared without any remedial action needed.

Graph 1 shows the PCV2 serology. There were no statistically significant differences between groups either for IgM or IgG at any age (p>0.1).

Graph 1. PCV2 IgG and IgM seroconversion



ELISA Ingezim PCV IgG index >0.520 and IgM index >0.66 – positive result

No clinical signs of PCV2 or *M.hyopneumoniae* infection were detected in any treatment group.

Discussion

This trial has demonstrated the compatibility of Porcilis® PCV and M+PAC® in terms of safety and the immune response against PCV2 IgG and IgM, even in the presence of maternally derived antibodies.

Although further studies will be needed to confirm efficacy in the field, these data suggest a way herd vaccination strategies might be simplified and animal welfare improved.

References

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EFFICACY OF DIFFERENT VACCINES AGAINST PORCINE CIRCOVIRUS TYPE2 ADMINISTERED AS SINGLE SHOT TO 3 WEEK-OLD PIGLETS WITH HIGH MATERNAL DERIVED IMMUNITY AGAINST PCV2

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Introduction

Infection of sows with PCV2 leads to varying antibody levels in blood and colostrum. High levels of maternal derived antibodies (MDA) can prevent PCV2 infection of piglets during their first weeks of life, but may interfere with the active immunization of piglets against PCV2 (*Palzer,IPVS 2010*). In recent years, several vaccines against PCV2 were brought to the market. All piglet vaccines can be given as a single shot vaccination from 2 or 3 weeks of age, when MDA can still be on a high level. Thus, it is essential that this single vaccination at young age is efficacious against possibly high levels of MDA. The objective of this study was to compare the efficacy of commercially available PCV2 vaccines in piglets with high MDA against PCV2 after a single vaccination at 3 weeks of age.

Material & Methods

Fifty piglets from sows with high PCV2 antibody titres (9.8 to 12.5 $\log_2$ ELISA units before farrow) were available for this study. Allocation of piglets to five groups of 10 was done across litters the way that average MDA titres per group were comparable. At three weeks of age one of the following PCV2 vaccines was injected:

Group 1 was vaccinated with 2 ml of Porcilis PCV(subunit vaccine), group 2 received 2ml of a PCV2 vaccine containing inactivated chimeric PCV1-2 virus as antigen, group 3 was injected with 1ml of a PCV2 subunit vaccine, group 4 received 0.5ml of an inactivated whole virus vaccine and group 5 was treated with 2ml of a physiological salt solution and served as negative control group. At 12 weeks of age, all piglets were challenge infected with wild type PCV2 challenge strain I-12/11 by the intranasal route.

All animals were observed daily for clinical signs and blood samples were taken on several time points. Three weeks post challenge all animals were necropsied and tonsil, mesenteric lymph node and lung were sampled for determination of PCV2 viral nucleic acid by Quantitative PCR (QPCR). Serology data at the time points >3 weeks were analysed by Analysis of Covariance (ANCOVA) by time point using the titre at time of vaccination and sow as covariate. Equality of serum antibody titre prior to vaccination was checked by ANOVA. Tukey's multiple comparison method was used to compare the treatments.

Results

No clinical signs were observed throughout the study. Serology: at time of vaccination the piglets had mean group titres of around 11 $\log_2$ ELISA units.

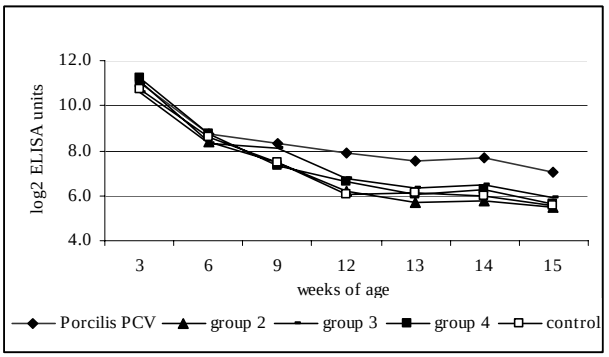

Fig 1: Development of PCV2 antibody titres

From week 9 post vaccination until the end of the study, the average antibody titre of piglets vaccinated with Porcilis PCV was significantly higher than in the other groups (Porcilis PCV/group 2 $p< 0.0014$; group 3 $p<0.0155$; group 4 $p< 0.0037$; control $p<0.0001$). No statistically significant difference in PCV2 antibody titres could be found at any time point between the other vaccinated groups or the unvaccinated controls.

QPCR on organs revealed that piglets vaccinated with Porcilis PCV had the lowest mean load of PCV2 DNA in all tested organs.

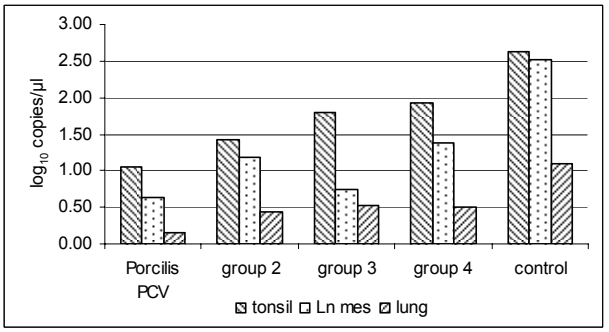

Fig. 2: Mean PCV2 nucleic acid load in organs

Conclusion

In this study, a single vaccination with Porcilis PCV induced a significantly higher humoral immune response in piglets with high MDA as compared to other PCV2 vaccines. The excellent protective efficacy of Porcilis PCV was confirmed by the low load of PCV2 DNA in all tested organs upon challenge infection.

References *Palzer, IPVS 2010*

EFFICACY OF CIRCOVAC® (MERIAL) ACCORDING TO DIFFERENT VACCINATION PROTOCOLS TO PREVENT PMWS IN A SPANISH PIG FARM

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Introduction

The objective of this study was to show how PCV2 sow vaccination with CIRCOVAC can improve mortality and runt rate over time and can lead to a control on the long run. CIRCOVAC was used in piglets as no other PCV2 piglet vaccine was registered.

Farm description

This study was run in a 450-sow farrow-to-finish Spanish operation with a 3-week farrowing batch management and a strict all-in/all-out pig flow. Pigs were weaned at 28 days of age and moved to the fattening barns at 13 weeks of age.

Case description - diagnosis

This farm was considered at high sanitary risk because of its location close to a busy road and the presence of a lot of concurrent infections (PRRSV, *Haemophilus parasuis*, *Streptococcus suis*, *Staphylococcus hyicus*). Post-weaning (PW) and fattening stages were depopulated in Apr 2006 and repopulated in Jan 2007. Pigs were affected with PMWS, porcine respiratory disease complex (PRDC) and diarrhoea. However, the diagnosis of PCVD was confirmed in Oct 2007 on 4 affected pigs submitted to laboratory examination (1): typical histopathological lesions were found and PCV2 presence associated with lesionned tissues was proved by *in situ* hybridization (ISH) technique. Although management practices were significantly changed in order to improve the sanitary conditions (reduction of herd size, partial depopulation in PW, change of batch management), the weaning-to-slaughter mortality and runt rate varied from 5.9 to 24%. Even if the fattening period was rather less affected with a mortality and runt rate close to 4 %, pigs from 4 to 13 weeks of age still exhibited the clinical signs described before.

Vaccination strategy and evaluation

At the start of PCV2 vaccination implementation, 1 batch of pigs (batch 7) was fully vaccinated at weaning with CIRCOVAC, 0.5 mL IM. Batch 8 was divided in 2 groups: 1 vaccinated and 1 left as control (8a, 8b).

Piglet vaccination was discontinued as soon as the 1st batch of piglets born from fully primo-immunized sows (i.e. 2 injections, 2mL IM, 5 and 2 weeks before farrowing) reached weaning. At the following gestation, sows received a booster injection, 2 mL IM, 2-3 weeks before farrowing. Replacement gilts were vaccinated as recommended (2 injections 2 mL IM, 5 and 2 weeks before breeding).

PW mortality and runts were recorded daily in every experimental batch.

Results

Figure 1: Mortality and runt rates at PW per batch depending on the protocols

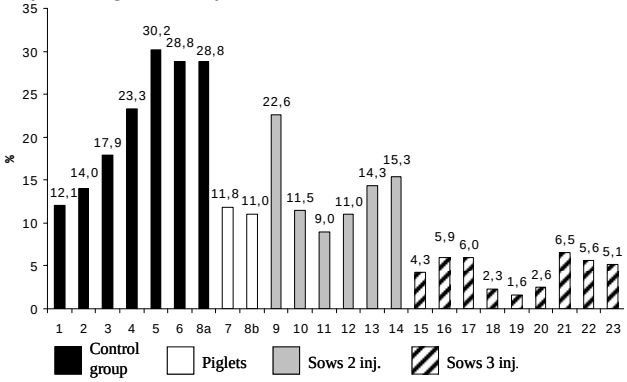

Table 1: Mean mortality and runt rates at PW depending on the protocols

Vaccination program	mean± STD
Control	22.14±7.52 [a]
Piglets	11.40± 0.56 [b]
Primo-immunized sows	13.95± 4.82 [b]
Boostered Sows	4.44± 1.82 [c]

[abc] Different supercripts in the same column mean significantly different values (Kruskal-Wallis test; p<0.05)
STD= Standard deviation

Discussion

Severity of mortality and runts in the outbreak clearly suggests a high virus pressure in the farm and probably especially around young piglets. Piglet vaccination induced a significant decrease of mortality and runts but still twice as high as in the situation of a full control of PCV2. Sow primo-immunization was not able to control mortality and runts in the first batch suggesting that PCV2 challenge in young pigs must have remained too high to be controlled by the primo-immunization of the dams at the beginning. However, quickly, mortality and runt rates went down at similar levels as in the vaccinated piglet batches. Furthermore, piglets born from sows having received the primo-immunization and a booster injection show mortality and runt rate that was consistently very low.

Conclusion

Interestingly, results of CIRCOVAC vaccination in sows dramatically improved over time after booster vaccination. Long term vaccination i.e. sows primo-immunization plus a booster stabilized mortality and runt rates twice as lower as piglet vaccination.

References

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SEROLOGY, PCV2 FECAL SHEDDING AND POST-WEANING GROWTH PERFORMANCE IN PIGLETS BORN FROM CIRCOVAC® VACCINATED SOWS IN VIETNAM

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Introduction

Post-weaning multisystemic wasting syndrome (PMWS) has been diagnosed in farms in Vietnam (1). This study aims to evaluate the efficacy of CIRCOVAC® by serology, growth, wasting and culling rates and the number of piglets shedding PCV2 virus in feces.

Material and Methods

The study was conducted in a 1200-sow farrow-to-finish commercial piggery in Vietnam. At 5 and 2 weeks prior to farrowing, 86 sows were injected with CIRCOVAC IM, 2ml; while 85 unvaccinated sows served as controls.

Piglets were individually weighed at birth, weaning (26 days of age) and 60 days of age. Mortality rate, stunting rate and health status were recorded.

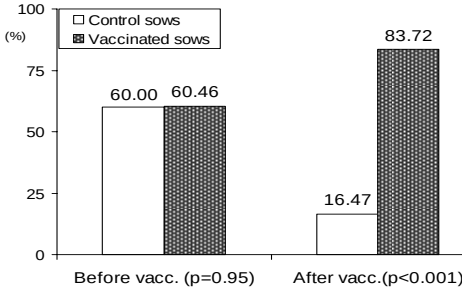
Sera from sows before the trial and one week after farrowing as well as piglets at 7 and 21 days old were titrated for PCV2 antibodies using a commercial ELISA test (SERELISA® PCV2 Ab Mono Blocking, Synbiotics Corp.) according to the method previously described (2).

Fecal samples at 21 days of age were tested for PCV2 antigen by ELISA tests (SERELISA PCV2 Antigen; Synbiotics Corp.). Maternal antibodies in piglets were evaluated by determining the number of piglets having PCV2 antibody titre higher than 4log10 ELISA (3). Statistical analysis was by Kruskal-Wallis test.

Results and Discussion

The number of vaccinated sows having seropositive titres after vaccination was significantly higher than that of the control group (Figure 1).

Figure 1: PCV2 antibody seroconversion (%) in sows



Significantly more piglets from vaccinated sows have PCV2 antibody titre higher than 4log10 ELISA at 1 week until weaning unlike the control group (Fig. 2). There were significant differences in growth between the 2 groups at birth, at weaning and at 60 days of age (Table 1). Results showed that significantly more piglets from the control group shed PCV2 via the feces (Table 2). Piglets from vaccinated sows had a significantly lower wasting/culling rate (Table 2).

Figure 2: Percentage of piglets with antibody titres >4log10 ELISA at 1 week of age and at weaning (26 days of age)

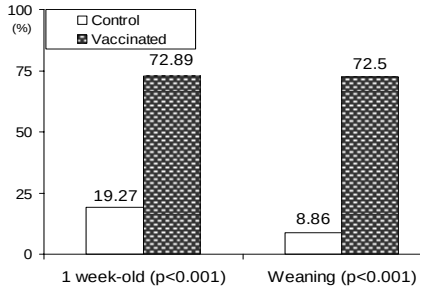


Table 1: Average body weight (kg) of piglets at birth, at weaning (26 days old) and at 60 days old

	At birth	26 days old	60 days old
Control	1.62±0.30	6.65±1.0	20.33±2.89
Vaccinated	1.69±0.33	7.11±1.08	21.37±2.37
Delta (kg)	+ 0.07	+ 0.46	+ 1.04
P value	0.000	0.000	0.000

Table 2: PCV2 fecal shedding and clinical signs in piglets

Results	Control group	Vaccinated group
No (%) weaners with PCV2 antigen in feces	[a]41.13% (65/158)	[b]9.37% (15/160)
N0. (%) wasted/culled piglets from weaning to 60 days of age	[a]3.76% (26/691)	[b]1.46% (10/685)
% piglets with clinical PMWS during the period post-weaning to 60 days-old	[a]3.18%	[b]0

[ab] Different superscripts in the same line mean significantly different values (p<0.01)

Conclusion

CIRCOVAC was effective at inducing an immune response in sows with transfer of passive immunity to the piglets which showed higher weight gains, lower wasting and culling rate at 60 days of age and lower number of piglets shedding PCV2 as compared to the control group.

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EFFICACY OF PCV2-VACCINATION WITH CIRCOVAC® (MERIAL) UNDER FRENCH CONDITIONS, A LARGE SCALE FIELD STUDY: REDUCTION OF MORTALITY

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Introduction

CIRCOVAC® piglet claim is not registered in Europe but it was used as no PCV2 piglets vaccine was registered in Europe. The objective of this study was to evaluate CIRCOVAC piglet vaccination through mortality rates during post-weaning (PW), finishing and both (weaning-to-slaughter) under field conditions in France.

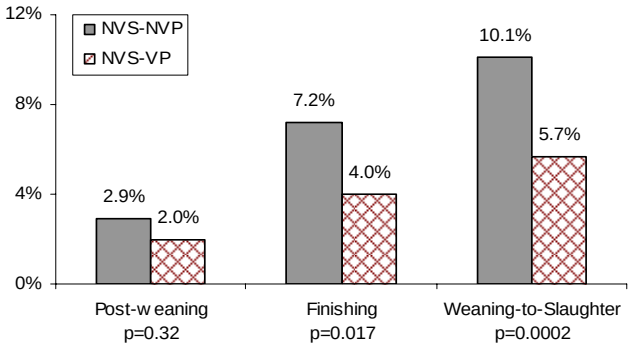
Material and Methods

Eighteen farms (63,500 piglets): 9 farrow-to-finish herds, 8 finishing herds and one wean-to-market herd were included in the study as they had the following characteristics: established diagnosis of PCVD (1), all-in all-out pig batches of no fewer than 100 pigs, unequivocal pig batch identification, reliable data recording system, and vaccination of pigs and/or sows for more than 6 months. Symmetry of the test was obtained comparing for each farm and vaccination regimen the same number of batches before and after vaccination. Experimental groups are defined as follows: vaccinated pigs from vaccinated sows (VS-VP), vaccinated pigs from non-vaccinated sows (NVS-VP), non-vaccinated pigs from vaccinated sows (VS-NVP) and non-vaccinated pigs from non-vaccinated sows (NVS-NVP). When vaccinated, sows were routinely injected with CIRCOVAC 2ml, IM either each batch 2-3 weeks before farrowing or mass-vaccinated 3 times a year. Piglets were vaccinated using CIRCOVAC 0.5ml, IM from 3 to 13 weeks of age. Criteria in this part of the study were PW, finishing and weaning-to-slaughter mortality rates compared before and after vaccination. Statistical analyses were performed using SAS® (SAS Institute Inc.) version 9.1.2 software. The experimental unit was the batch of pigs. Statistical significance was for $p<0.05$.

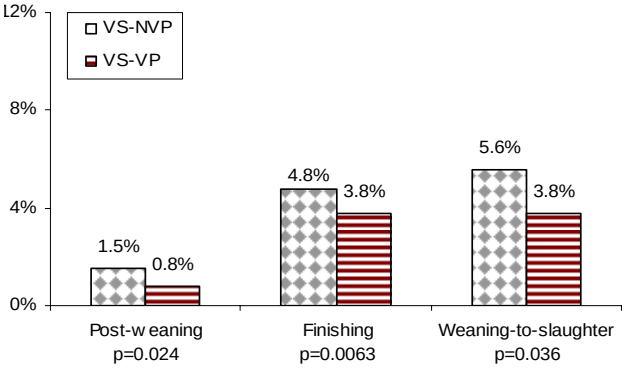
Results

Figure 1: Mortality rates in the 4 experimental groups

a) Piglets from non-vaccinated sows



b) Piglets from vaccinated sows



Discussion

PCV2 vaccination of sows or piglets as well as sows plus piglets significantly decreased mortality both in PW and in finishing (except piglets born from non-vaccinated sows in PW). In this study, piglet vaccination added to sow vaccination showed a decrease of 1.8 percentage points compared to sow vaccination "only". Vaccination of sows has been shown to improve the pre-weaning mortality (2), and in addition piglet vaccination will immunize pigs that did not receive enough colostrum in their 1st hours of life and will logically complement sow vaccination benefits.

One can see that the levels of mortality reached by piglet vaccination "only" are roughly the same as those obtained with sow vaccination "only". Vaccination of piglets plus sows decreased mortality even more, although to a slight extent, as it is expected to compensate the lack of proper colostrum intake in some of the pigs and to protect piglets against immunocompromization that would prevent them to mount a proper immune response to PCV2 active immunisation.

Conclusion

In this study, vaccination of piglets born to vaccinated sows significantly decreased PW, finishing and weaning-to-slaughter mortality rates. Vaccination of piglets born to non-vaccinated sows decreased mortality rates in the 3 stages, significantly in finishing and from weaning to slaughter.

References

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2. Joisel F. *et al.*, (2007) 5th International symposium on emerging and re-emerging pig diseases, Krakow, Poland, p126

CASE STUDY: EFFECT OF TEMPORARY PIGLET AND LONG-TERM SOW PCV2 VACCINATION ON THE PRODUCTIVITY IN A FARROW-TO-FINISH HERD IN FRANCE

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Introduction

The objective of this study was to confirm that PCV2 vaccination with CIRCOVAC® (Merial) using initial implementation of both sow and piglet vaccination could improve the productivity of a herd affected by PCVD.

Farm description

The study was carried out in an 85-sow farrow-to-finish farm in the centre of France. Piglets are weaned at 28 days of age and moved to the finishing barns after an 8 to 9-week period of time. Piglets are fattened during 16 weeks on average. Hygiene and management were average and no changes occurred through out the course of the study.

Case description – Diagnosis

The study lasted for 3 years; 2006 to 2008. Since 2005 poor growth in post-weaning and fattening had been observed along with low pre-weaning piglet weight due to diarrhea. A vaccination against clostridial diarrhea improved the diarrhea status of the piglet, but growth remained poor in post-weaning and mainly in fattening. Late finishing PCVD resulted in increased runt and late mortality rates including cases of porcine dermatitis and nephropathy syndrome (PDNS).

PCV2 vaccination and nutrition strategies

In February 2007 sows were vaccinated with CIRCOVAC, batch after batch at 6 and 3 weeks before farrowing, and gilts received 2 injections 3-weeks apart in quarantine. Sows and gilts received a 3-week pre-farrow booster at every subsequent gestation. At the same time piglets were vaccinated at 6 weeks of age with 0.5 ml CIRCOVAC, IM. Piglet vaccination was stopped when piglets from fully vaccinated sows reached weaning age. In late February 2007 the use of a new wheat stock led to suspect a mycotoxin contamination because of low feed consumption and still poor growth rates. Deoxynivalenol (DON) presence was Lab confirmed (DON>5,000ppb) and lasted until change of cereal source in July. Cereal quality monitoring through inspection and Lab analysis confirmed that no DON issue was seen before late February 2007, nor after July 2007. Due to the irregular nutrition and the fact that vaccination was implemented within 2007, "Before and after" vaccination results have to be compared in full years 2006 *versus* 2008.

Results and discussion

Year 2006 can be considered as a full year "before vaccination". Productivity results began to improve in year 2007, even though the DON contamination was regarded as having impaired pig growth and as having induced a decrease in production results. That is why this "vaccination set-up year" 2007 was chosen not to be taken in account in the comparison. After changing pig feed in July 2007, the productivity results were back to the good levels expected after PCV2 vaccination.

Table 1: Productivity results from 2006 to 2008

		2006	2007	2008	Δ2008-2006 (%)	2008 - 2006
Productivity	Kg liveweight produced/present sow/year	1,723	1,840	1,859	+7.9	+136 kg
	Feed consumption/present sow /year	1,133	1,068	1,058	-6.6	-75 kg
	Age at 25kg (days)	82	67	60	-26.8	-22 days
	Age at 115kg (days)	214	200	178	-16.8	-36 days
	Average weaning weight	7.2	7.5	7.8	+8.3	+0.6 kg
Weaning-to-slaughter results	Average final weight	112.5	117.4	116.6	+3.6	+4.1 kg
	Mortality and non-marketable pigs	8.5	5.5	4.91	-42.2	-3.59 pts
	ADWG from 8 to 115 (g)	571	617	703	+23.1	+132 g
	Days on feed	183	178	152.7	-16.6	-30.3 days

Therefore comparison was established between 2006 and 2008 as the full year "during vaccination". Despite of the lateness in improvement due to DON contamination, productivity results have gradually improved. The increase in the weaning weight from 7.2 to 7.8 that is most likely due to sow vaccination with CIRCOVAC also confirms results obtained in other studies (1). As weaning weight has a crucial importance for further optimal growth (2), this vaccination strategy, that allies immediate results with short-term piglet vaccination along with long-term improvement with sow vaccination for an acceptable cost is highly valuable.

Conclusion

Both short term piglet vaccination with CIRCOVAC associated with long term sow vaccination provided major productivity improvements in the farm.

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EFFICACY OF DIFFERENT VACCINATION PROTOCOLS AGAINST POST-WEANING MULTISYSTEMIC WASTING SYNDROME (PMWS)

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Introduction

CIRCOVAC® piglet claim is not registered but was tested as no PCV2 piglet vaccine was registered in Europe. The objective of this study was to compare the clinical efficacy of 3 different CIRCOVAC vaccination programs under Polish conditions.

Materials and Methods

The study involved a 2,550-sow and 30-boar farrow-to-finish herd, PMWS was diagnosed following the criteria of the EU PCV2 consortium (1). CIRCOVAC vaccination started in December 2007. Three different vaccination protocols were compared in 5 groups of 6 consecutive batches. Vaccination of sows (2 injections 2ml CIRCOVAC IM, 3 weeks apart before farrowing), vaccination of piglets (1 injection 0.5ml IM at weaning, 28 days of age) and vaccination of sows and piglets (piglets vaccinated at 7 weeks) were compared with 1 group of 6 non-vaccinated batches during PMWS and 1 group of 6 non-vaccinated batches before the outbreak.

Table 1: Experimental groups

Group	Nb of sows	Nb of piglets	Vacc. program
before PMWS	651	6,894	None
during PMWS	628	6,169	None
S	636	6,838	Sows
P	653	7,187	Piglets
SP	608	6,058	Sows+Piglets

Statistic analyses used SYSTAT® 5.0 computer program (SYSTAT Software Inc.). Barlett test, ANOVA (or Kruskal Wallis one-way test if no homogeneity of variance) and Tukey's test were used. The level of significance was 5% for all the tests.

Results

Table 2: Progeny performance in the 5 groups

	ADWG (g)	Mortality at 1 month of age (%)	Total mortality (%)	Slaughter weight (kg)
before PMWS	^{a}611.2 ± 9.09	^{a}6.22±0.49	^{a}17.29±1.48	^{a}94.8±1.56
during PMWS	^{b}568.5 ± 7.18	^{bc}5.26±0.31	^{b}28.76±4.89	^{b}92.5±1.16
S	^{c}635.2 ± 6.24	^{bd}4.77±0.26	^{c}16.93±0.63	^{c}100.9±1.35
P	^{d}640.3 ± 3.50	^{ac}5.60±0.39	^{d}16.12±0.90	^{d}98.1±0.54
SP	^{e}656.0±12.22	^{d}4.55±0.53	^{e}15.35±1.35	^{e}98.6±2.32

^{abcde} Different superscripts in the same column mean a significant difference ($p<0.05$).

No adverse reaction was noted. The PMWS outbreak caused significant decreased ADWG and increase in total mortality. But all production parameters significantly improved after vaccination and values were as good as before PMWS, or even better: ADWG (see Table 2).

Discussion

All 3 protocols proved efficacious in PMWS control. Sow vaccination significantly decreased pre-weaning mortality. It has been well established that in pigs further growth is related to health status and weight at weaning (2). This confirms previous results showing that sow PCV2-vaccination was leading to an increase of pre-weaning ADWG (3). Consequently, pigs gained slaughter weight in shorter fattening period. Sow vaccination coincided also with increase of conception rate of about 7% (not shown). As no laboratory analysis regarding reproduction was made, it can be only speculated that this effect was seen due to control of sub-clinical infections in sows. The highest ADWG in group SP may have several explanations. When only sows are vaccinated, some pigs may not get the sufficient amount of colostrum and when piglets only are vaccinated, in acute outbreak, with a high virus pressure, some of them may be immuno-compromised to such a degree that this will impair the active immunization. A second explanation may also sit in the fact that after 6 batches of vaccinated sows and 6 batches of vaccinated piglets, PCV2 pressure may have decreased and become less detrimental to the immune balance of the herd and the growth performances. So when both are vaccinated, it can be expected that growth performance is optimized.

Conclusion

In this farm acutely affected with PMWS, the efficacy of PCV2 vaccination was confirmed. The 3 vaccination programs gave good improvement. The better growth was obtained using sow plus piglet vaccination. Some parameters turned out to be even significantly better than before PMWS outbreak, suggesting that a sub-clinical impact of PCV2 may be underestimated. However, before implementing such solution in farms, benefits should be analyzed case by case versus vaccine and labor costs.

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EFFICACY OF PIGLET VACCINATION WITH ONE-SHOT CIRCOVAC® 0.5ML I.M. IN THREE GERMAN COMMERCIAL FARMS

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Introduction

Since the first reports on porcine Circovirus type 2 (PCV2) mid of the 1990s, PCVD became a major problem world-wide. Efficacy of sow vaccination with CIRCOVAC® (Merial) has been extensively proven since the registration in the European Union in 2007 (1). Since May 2008 piglet vaccination with CIRCOVAC has been performed under special authorisation according to §17c.Abs.2 TSG in Germany. This paper presents case studies of three different commercial farms showing the efficacy of CIRCOVAC piglet vaccination on performance parameters.

Material and Methods

The 3 farms represent different farm categories resp. sizes. Farm A is a piglet producer, B and C are farrow-to-finish units (Table 1). Data of performance parameters before and with piglet vaccination were collected per vaccinated batch via questionnaires provided by Merial to the veterinarians. Data were available and analysed for nursery pigs **(N)** in all farms and fattening pigs **(F)** in farms B and C.

Table 1: Production type and farm size

Farm	N° of sows	N° of weaners	N° of fatteners
A	80	400	-
B	750	2960	3440
C	40	120	240

On farm A PCV2 vaccination was requested by the piglet marketing organisation; no PCV2 diagnostic was performed prior to vaccination. On farm B vaccination was implemented due to PCVD problems. PCV2 diagnosis was approved by means of clinical signs and positive results for PCV2 by qPCR and serology. No respective information was available for farm C. Mortality rate, average daily weight gain (ADWG), percentage of wasting animals in all farms and additionally in farm B percentage of PDNS were recorded and compared before and during vaccination. Mortality, wasting and PDNS rates were analysed using z-test, ADWG using t-test.

Results

In all farms mortality and wasting rates in nursery period were significantly decreased. A significant difference occurred also on farm C during fattening period. Results are displayed in tables 2 and 3.

Table 2: Mortality rates (%) before and with piglet vaccination

	Farm	Before	With vacc.*	Delta
N	**A**	4.2	1.5 ±0.59	-2.7***
	B	6.0	4.57 ±1.19	-1.43**
	C	5.0	0.32 ±0.11	-4.68***
F	**B**	4.3	3.28 ±0.81	-1.02[a]
	C	3.0	0.16 ±0.06	-2.84***

*Mean over all groups per farm ± standard deviation (SD); **p<0.05; ***p<0.0001; [a]p=0.066

Table 3: Percentage of wasting animals (%) before and with piglet vaccination

	Farm	Before	With vacc.*	Delta
N	**A**	7.2	1.3 ±0.83	- 5.9***
	B	4	2.5 ±0.42	- 1.5**
	C	2	0.2 ±0.11	- 1.8***
F	**B**	1	0.7 ±0.25	- 0.3[a]
	C	1	0.1 ±0.05	- 0.9***

*Mean over all groups per farm ±SD; **p<0.05; ***p<0.0001; [a]p=0.162

ADWG was significantly increased on all farms during nursery and fattening (Table 4).

Table 4: ADWG (g/day) before and with piglet vaccination

	Farm	Before	With vacc.*	Delta
N	**A**	384.0	451.0 ±17.55	+ 67.0***
	B	380.0	393.3 ±11.79	+ 13.3***
F	**B**	720.0	765.6 ±33.04	+ 45.6***
	C	750.0	827.3 ±10.87	+ 77.3***

*Mean over all groups per farm ±SD; ***p<0.0001

In farm B, PDNS was significantly reduced from 1.0% to 0.28% (N) and from 1.0% to 0.41% (F).

Discussion and Conclusions

Piglet vaccination with CIRCOVAC led to significant improvement of the performance parameters mortality rate, wasting rate and ADWG in nursery (farm A, B, C) and fattening period (farm C). In Farm B, additionally to significant improvement of ADWG in nursery and fattening periods, PDNS was significantly reduced in both periods.

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IMPROVEMENT OF REPRODUCTIVE PERFORMANCE IN SOWS AFTER CIRCOVAC® VACCINATION

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Introduction

Recent studies have shown that PCV2 is involved not only in PMWS, but also in several other disease presentations, including reproductive disorders, deteriorating sow performance (1, 2). The objective of this study was to assess the impact of CIRCOVAC® on the reproductive performance of a sow breeding unit weaning pigs on-site and selling them at 35kg.

Material and Methods

A 1000-sow outdoor breeding unit started vaccinating the breeding animals in May 2007 with a PCV2 inactivated vaccine, CIRCOVAC. The first vaccinated sows farrowed in July 2007. PCVD has been clinically diagnosed on the nursery and finishing units. The number of pigs born alive per litter and weaned per litter was routinely recorded using herd management software.

Historical data, since 2005 were collected. The data were divided into blocks of 6 months from the start of vaccination for the periods before and after. The data were also compared for a period of 1 year before and after PCV2 vaccination was implemented. Statistical analyses were undertaken using the two sample t-test with unequal variances and a two-factor (month and year) analysis of variance. Tests were carried out using proprietary statistical software.

Results

Live born piglets per litter after CIRCOVAC vaccination were increased by 1.425 pigs, $p<0.001$ (t-test) compared to the period before (2004 to July 2007).

Analyzing the data before and after July 2007 by a t-test shows that there is an improvement in the mean number of pigs of 1.066 pigs weaned per sow per litter after CIRCOVAC vaccination started.

Regarding pigs weaned per sow per litter, (Table 1) there was no significant difference between the first 6 months of 2005, 2006 and 2007 (before vaccinated sows started farrowing) but these were significantly different to the first 6 months of 2008 and 2009 (CIRCOVAC vaccinated sows started farrowing in July 2007), $p<0.001$. Similarly that there was no significant difference between the second 6 months of 2004, 2005 and 2006 (before vaccinated sows started farrowing) but these were significantly different to the second 6 months of 2007 and 2008.

The use of CIRCOVAC on sows resulted in an improvement of 0.8 pigs born alive between the 6 months before the use of vaccine and 6 months after, from 10.27 to 11.07 respectively.

Table 1: Six month periods with mean number pigs born alive per litter

	January – June	July - December
2004		9.901^{a}
2005	10.07^{a}	10.048^{a}
2006	10.2^{a}	9.78^{a}
2007	10.27^{a}	11.07^{b}
2008	11.69^{b}	11.24^{b}
2009	11.82^{b}	

Different superscripts (a,b) indicate significant difference $p<0.001$

The number of piglets weaned per sow per litter was significantly different between the first half of the years 2005, 2006, 2007 and the first half of 2008 and 2009, the mean number of pigs weaned per litter for the periods of July to December 2004 and 2006 was significantly different to the same periods in 2007 and 2008, Table 2.

Table 2: Six month periods with mean number pigs weaned per litter.

	January – June	July - December
2004		8.892^{a}
2005	9.672^{a}	9.22
2006	9.847^{a}	8.685^{a}
2007	9.842^{a}	10.07^{b}
2008	10.767^{b}	10.14^{b}
2009	10.923^{b}	

Different superscripts (a,b) indicate significant difference $p<0.001$

Discussion and conclusion

This study shows the benefits of using CIRCOVAC on the reproductive performance of vaccinated sows.

Improving the number of pigs weaned per sow per litter optimizes the sows' performance. This increase reduces the cost of production and considerably increases profitability (3).

The constant number of pigs born alive and weaned per litter during the post vaccination period indicates that the improvement achieved with CIRCOVAC vaccination resulted in a stable and sustained increase of the number of piglets born alive and weaned per litter.

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IMPACT OF CIRCOVAC® SOW PCV2 VACCINATION ON PIGLET WEANING WEIGHT

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Introduction

Early exposure to PCV2 compromises the ability to respond to infectious agents (viral and bacterial) if the pig is exposed to PCV2 without the presence of protective passive immunity. The objective of this study was to evaluate the effect of CIRCOVAC® sow vaccination on the pre-weaning performance of the pigs.

Material and Methods

The chosen farm was a 1,270-sow indoor breeding unit farrowing 60 sows per week and weaning off site.
PCVD haven't been previously diagnosed on the progeny of this breeding unit and no PCV2 vaccine was being used before this study.
Dam line gilts were weaned before the weighing and were not included in this study.
The average weaning age before and after was approximately 28 days. Sows started being vaccinated with CIRCOVAC in June 2008, following the recommended protocol.
To evaluate the impact of PCV2 sow vaccination on piglet weaning weight, 1,007 piglets from non-vaccinated sows and 955 pigs born from vaccinated sows were weighed at weaning. The skewness and kurtosis values of the weights at weaning were studied following a normal distribution (at 95%) and the live weaning weight (LWW) before *versus* after vaccination was compared using the following statistical tests, Bartlett test, student t-test and Kruskal-Wallis one way analysis of variance.
The distribution of pigs in different weaning weight groups was also evaluated using chi^2 test.

Results

Table 1: Evaluation of the weaning weights before and after sow vaccination

	Before vaccination (n=1,007)	After CIRCOVAC vaccination (n=955)	Delta
Mean	7.73	8.66	0.93 p<0.001
std	1.486	1.424	

The number of total pigs born alive per litter for a period of 35 weeks before and after CIRCOVAC sow vaccination started, was 11.1 and 11.3, respectively. The number of pigs weaned on average per litter for the same period was 9.5 and 9.4, respectively. This was due to the PRRS problems that affected the sow herd and impacted in the general sow health.
The results of the analysis of the data collected can be seen on table 1. A significant improvement in the average weaning weight after the use of vaccine of 0.93 Kg was showed (student t-test and Kruskal Wallis tests; p<0.001 and <0.001 respectively).
The percentage of pigs weighing less than 6, 7 or 8 kg of LWW can be seen on table 2.

Table 2: Percentage of pigs weighing less than 6, 7 or 8 kg of LWW

	Before Vaccination (n=1,007)	After CIRCOVAC Vaccination (n=955)
% pigs < 6kg LWW	12.1[a]	1.8 [b]
% pigs < 7kg LWW	33.5[a]	11.6 [b]
% pigs < 8kg LWW	56.4[a]	32.8 [b]

[a,b] Different superscripts in the same row mean significant difference (Chi 2 test; p<0.001).

Discussion

Lighter weaning weights and percentage of small pigs are associated with higher mortality and slower growth. Heavier pigs perform better from weaning to slaughter, result of higher daily live weight gains (1, 2).
A significant improvement in the mean weaning weight of 0.93 kg was observed in this study.
Reduction of the number of pigs with a low weaning weight have the advantage of making multi-site systems easier to manage (2) and may also represent a direct financial improvement to the breeding herd as some weaner producers receive premiums based on weaning weights.
In this study, the use of CIRCOVAC resulted in the reduction of the percentage of pigs weighing less than 6 kg from over 12.1% to 1.8%. The improvements observed may be attributed to the protection granted by maternally derived immunity against PCV2 challenge, which improves the health of the animals from birth, improving the growth of the animals, resulting in a higher weight at weaning.

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DURATION OF IMMUNITY AFTER VACCINATION OF PIGS AT MORE THAN 96 DAYS OF AGES WITH RESPIPORC® FLU3 (IDT)/GRIPOVAC® 3 (MERIAL)

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Introduction

The prevalence of swine influenza viruses may be high with 60% of farms affected (1). Previous studies showed an increase in the prevalence of the H1N2 subtype (2) contained in the vaccine RESPIPORC® FLU3/ GRIPOVAC® 3.
This paper summarizes a study aiming at showing the duration of immunity (DOI) of RESPIPORC FLU3/GRIPOVAC 3 vaccination in pigs vaccinated after 96 days of age.

Material and Methods

Pigs were free of antibodies against H1N1, H1N2 and H3N2. The study was blinded, placebo controlled and randomised. A challenge was performed at more than 6 months after basic immunisation by aerosol nebulisation with heterologous strains from Central Europe isolated from pigs affected with severe respiratory illness as described in table 1.

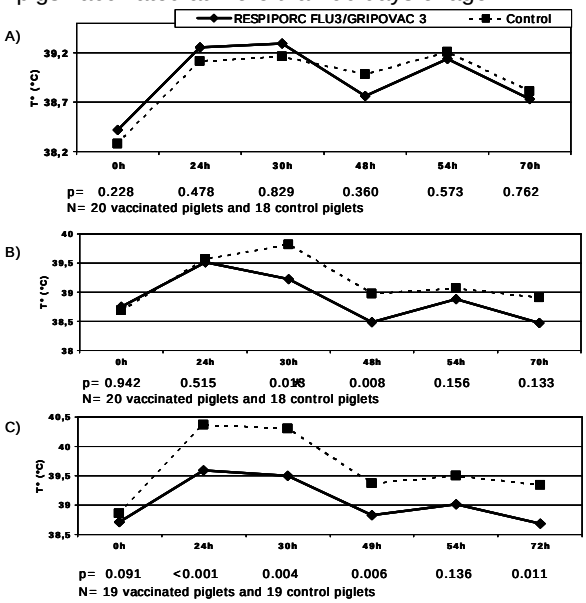
Table 1: Study parameters

Subtype	H1N1	H1N2	H3N2
No of pigs	40	40	40
Vaccinated	20	20	20
Control	20	20	20
Challenge	$10^{6.25}$ $TCID_{50}$/ml Aerosol 211d after 2nd vacc	$10^{6.5}$ $TCID_{50}$/ml Aerosol 211d after 2nd vacc	$10^{8.75}$ $TCID_{50}$/ml Aerosol 209d after 2nd vacc

Vaccination schedule: pigs were vaccinated at least at 96 days of age (including maes and females) 2 ml, twice 3 weeks apart. Control pigs were injected with NaCl 0.9%.
Criteria of data collection: Temperature was measured twice a day. Dyspnoea scoring: 1= enhanced abdominal breathing; 2= severe abdominal breathing; 3= strong breathing covering the whole body.

Results and discussion

Figure 1: T° after challenge A)H1N1, B)H1N2, C)H3N2 in pigs vaccinated at more than 96 days of age.



Globally, the clinical signs are lower in pigs vaccinated at more than 96 days of age and challenged 6 months after vaccination (figure 1, tables 2-4).

Table 2: Dyspnoea scores after challenge H1N1

	24h	30h	48h	54h	70h
RESPIPORC FLU3/GRIPOVAC 3	0.88	0.5	0	0.1	0
Control	1.67	0.81	0.19	0.31	0
p	0.005	0.360	0.408	0.460	1.000

N= 20 vaccinated piglets and 18 control piglets

Table 3: Dyspnoea scores after challenge H1N2

	24h	30h	48h	54h	70h
RESPIPORC FLU3/GRIPOVAC 3	1.35	1.1	0.2	0.2	0
Control	1.86	1.5	1.22	0.5	0.28
p	0.228	0.356	0.002	0.243	0.243

N= 20 vaccinated piglets and 18 control piglets

Table 4: Dyspnoea scores after challenge H3N2

	24h	30h	49h	54h	72h
RESPIPORC FLU3/GRIPOVAC 3	0.47	0	0.22	0.06	0
Control	2.08	1.78	1.39	1.22	0.56
p	0.002	<0.001	<0.001	0.001	0.113

N= 19 vaccinated piglets and 19 control piglets

Conclusion

Pigs vaccinated with RESPIPORC FLU3/GRIPOVAC 3 after 96 days of age were protected against the 3 strains from 1 week up to 6 months after the 2nd vaccination.

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FIELD SAFETY OF THE ADMINISTRATION OF A NEW SWINE INFLUENZA VACCINE: RESPIPORC® FLU3/ GRIPOVAC® 3 IN PREGNANT SOWS AND GILTS

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Introduction

The prevalence of Swine Influenza viruses may be high with 60% of farms affected (1). Previous studies showed an increase in the prevalence of the H1N2 subtype (2) contained in the vaccine RESPIPORC® FLU3/ GRIPOVAC® 3.

The objective of these studies was to confirm the safety of RESPIPORC® FLU3/GRIPOVAC® 3 vaccination during gestation in the field.

Material and Methods

A total of 107 sows and gilts from several farrow-to-finish conventional farms were used at different stages of pregnancy (see Table 1).

Table 1: Experimental design

	Sows T1	Sows T2	Sows T3	Gilts
Total sows in farm	206	530	200	530
Vaccinated sows	12	14	12	17
Control sows	12	16	11	13

T1=1st third of pregnancy. T2=2nd third of pregnancy. T3=3rd third of pregnancy.

Vaccination schedule

T1 sows: 1st injection at D0=from day 1 to day 38 of pregnancy, and 2nd injection at D21.

T2 sows: 1st injection at D0=from day 39 to day 78 of pregnancy, and 2nd injection at D21.

T3 sows: 1st injection at D0=from day 76 to day 114 of pregnancy, and 2nd injection at D21.

Gilts: 1st injection at D0= 5 weeks before insemination, 2nd injection at D21, 3rd injection at D133.

Safety criteria were rectal body temperature, local reactions i.e. skin colour, swelling, abscess, and systemic reactions i.e. behaviour, respiration, digestion, skin, coughing, others. Clinical status was monitored every day throughout the study.

Clinical observation: T1, T2 and T3 sow temperature was measured on D0, D0+4h, D1, D2, D21, D21+4h, D22 and D23. In gilts it was measured on D0, D0+4h, D1, D2, D21, D21+4h, D22, D23, D133, D133+4h, D134 and D135.

The local reactions have been monitored at the same time points and on D106 in T1 sows, on D100 in T2 sows, on D64 in T3 sows and on D177 in gilts, i.e. the last day of the study in each case.

Statistical analyses: An equivalence version of the t-test was used to compare the absence of difference between temperatures. The level of significance was 5%.

Results

Figure 1: T1 temperature curves *versus* control (equivalence version of the t-test: p< 0.0007)

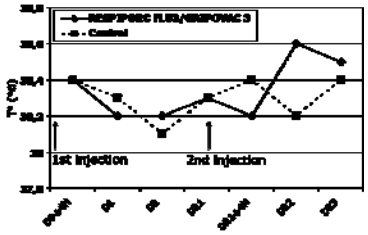


Figure 2: T2 temperature curves *versus* control (equivalence version of the t-test: p< 0.0003)

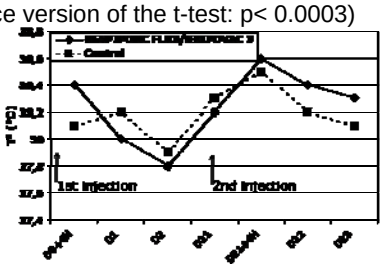


Figure 3: T3 temperature curves *versus* control (equivalence version of the t-test: p< 0.0011)

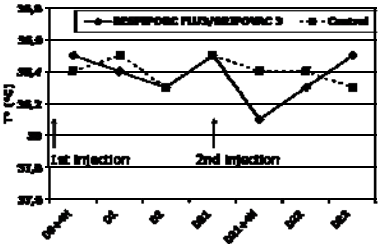
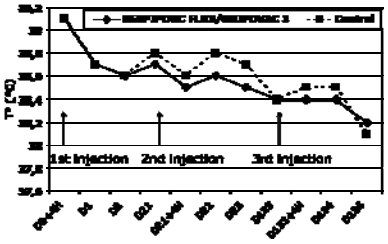


Figure 4: Gilts temperature curves *versus* control (equivalence version of the t-test: p< 0.0001)



Neither relevant local nor systemic reactions were noticed. The temperature is significantly non-different both in vaccinated from control groups.

Conclusion

The use of RESPIPORC® FLU3/GRIPOVAC® 3 is perfectly safe in pregnant sows and gilts, any time during gestation.

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SEROPREVALENCE OF H1N1, H3N2 AND H1N2 INFLUENZA VIRUS IN 29 PIG FARMS IN FRANCE IN 2009

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Introduction

Swine influenza viruses (SIVs) represented by three different subtypes, H1N1, H3N2 and H1N2 are now co-circulating in European countries (1). In France, according to recent virological and serological surveillance studies, H1N1 and H1N2 are the major subtypes and no or very few H3N2 viruses have been evidenced (2,3).
The objective of this survey was to evaluate the seroprevalence of SIVs in farms affected with acute respiratory clinical signs in France.

Material and methods

Experimental design: 29 farms (2 farrowing units, 17 farrow-to-finish and 10 finishing herds) located mostly in Brittany and western part of France were investigated. These farms had been affected with acute respiratory clinical signs at least 3 weeks before sampling. Four farms used to vaccinate the sows against H1N1 and H3N2 strains; in these specific cases, only H1N2 serological results were considered on sow sera. Farms were sampled from January to June 2009 as follow: 10 sows of different parity in farrowing units, 10 sows and 10 fattening pigs before slaughter in farrow-to-finish farms and 10 pigs before slaughter in fattening farms. In one farm, where fattening pigs showed fever, nasal swabs were collected for virus isolation. All sera and swabs were sent to IZLER laboratory at Parma, Italy, to be submitted to haemagglutination inhibition tests (HI) (4) and to virus isolation. The strains used for the HI test were H1N1 sw/Belgium/1/98, H1N2 sw/Scotland/41440/94, H1N2 sw/France/134240/09 (strain isolated on nasal swabs from one farm in Brittany during the study) and H3N2 sw/Gent/84. The results were analyzed according to the following rules: samples were considered positive for one specific subtype if the titre is ≥1/20; one farm was classified as positive against one given subtype if at least 2 sera showed an HI titre ≥1/20 against this subtype. Risks of misinterpretation due to cross-reactivity between H1N2 and H1N1 strains were decreased by considering negative for H1N2 a serum that showed higher (3 log2) HI titre against H1N1.

Results and discussion

The serological results are presented in table 1. Globally, 96.6% of farms tested were positive for at least one SIV subtype. The use in the HI test of the strain H1N2 sw/France/13240/09, isolated in Brittany, increased the sensitivity of the HI test, as showed in the table 2. This observation confirms the importance to use local strains in HI tests in SIV surveillance programme. Some farms were infected with both strains H1N1 and H1N2: this represents 38% of tested farms. One farm not vaccinated against flu was found to show positive sera against H3N2, and another one was doubtful. H3N2 subtype infection has not been shown since many years in pigs in Brittany, the possibility that cross reactions could have been occurred must be considered.

Table 1: positive serological results by HI tests (%)

Tested strain	N° farms tested	% positive farms
H1N1 sw/Belgium/1/98	29	72.4
H1N2 sw/Scotland/41440/94	29	13.8
H1N2 sw/France/134240/09	29	58.6
H3N2 sw/Gent/84	29	3.4*

*1 farm only was found positive

Table 2: H1N2 results in one farm: comparison of positive samples by strain used for the HI test.

Titre	<20	20	40	80	160	total
H1N2 sw/Scotland/ 41440/94	20	0	0	0	0	20
H1N2 sw/France/ 134240/09	4	1	7	3	5	20

Conclusion

This serological survey confirms the major role of SIVs in swine respiratory disorders in French farms. SIV of H1N1 and H1N2 subtypes are enzootic in swine producing region of Brittany, particularly about 50% of tested farms were found to be infected by H1N2 subtype when tested with a strain representative of the strains circulating in the region.

Acknowledgements: The authors wish to thank all practitioners who participated in this survey.

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EVALUATION OF THE SAFETY OF AN INACTIVATED PORCINE CIRCOVIRUS TYPE 2 VACCINE (CIRCOVAC®) ADMINISTERED TO BOARS: ABSENCE OF IMPACT ON SPERMATOGENESIS

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Introduction

Porcine circovirus type 2 diseases (PCVD) have been reported throughout the world as a major cause of losses in pig herds (1). PCV2 can clearly be associated with some cases of reproductive failure. Furthermore, the presence of infectious PCV2 in semen has been demonstrated (2,3,4). Consequently, PCV2 boar vaccination is commonly used in pig farms. This study was designed to assess the safety of CIRCOVAC® administered to reproductive boars.

Material and Methods

The study was performed in a European artificial insemination center. A total of 15 9-month-old boars provided by the same nucleus farm were included in the study and randomized as follows: 5 boars vaccinated twice 3 weeks apart with CIRCOVAC, 2 ml (group 1); 5 boars vaccinated according to the same protocol and treated with paracetamol dose for 3 days since the day before vaccination (group 2); 5 boars non vaccinated but injected with NaCl 0.9%, 2 ml twice (group 3). Boar semen was sampled once a week from 3 weeks before 1st vaccination to 8 weeks after 2nd vaccination. The criteria followed up were: body temperature and semen quality (volume, spermatozoon concentration, motility, abnormality, viability and healthy spermatozoon per ejaculate meaning mobile, with normal mobility and non agglutinated). A one-way analysis of variance (ANOVA) was used to compare temperature results between groups. Semen parameters were compared by a Student t-test after pooling both vaccinated groups 1 and 2 (vacc.) which were similar.

Results and Discussion

Body temperature was slightly increased after vaccination (4 boars out of 5 were above 39.5°C in group 1); this was limited to 1 and 3 boars respectively after 1st and 2nd injection in group 2 treated with paracetamol.

Semen quality parameters for each period (before, during and after vaccination) for the vaccinated and control groups are presented in table 1. No group effect was evidenced whatever the period ($p>0.05$). Only one boar in group 2 had a lower semen concentration during vaccination period. The statistical analysis only evidenced higher values for spermatozoon concentration and viability for vaccinated groups.

Very few impact of CIRCOVAC vaccination was observed in this study on semen quality parameters over a period of 10 weeks after 1st injection.

Conclusion

CIRCOVAC vaccination showed no impact on spermatogenesis as far as semen parameters of reproductive boars are concerned.

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Table 1: semen parameters according to the treatment group (mean value ±standard deviation)

Semen parameters	**Concentration (Million / ml)**		**Healthy spz (Billion / ejaculate)**		**Viability (%)**	
Groups (Nb of boars)	**V** (10)	**C** (5)	**V** (10)	**C** (5)	**V** (10)	**C** (5)
Before vaccination (5 weeks)	201.9± 31.2	180.4± 40.8	47± 13.1	45.7± 25.0	85.5± 2.1	86.4± 1.6
During vaccination (4 weeks)	246.5± 62.6	213± 36.4	60.3± 14.1	51.2± 22.4	86.1± 1.8	85.3± 3.1
After vaccination (6 weeks)	201.7± 40.6	191.9± 36.9	59.5± 12.5 (9 boars)	49.5± 18.3	85.1± 2.6	85.1± 2.4

V: vaccinated; C: controls

EVALUATION OF INF-γ SECRETING CELL RESPONSE AFTER *IN VITRO* VIRAL RECALL BY USING HOMOLOGOUS AND HETEROLOGOUS ISOLATES IN PIGS VACCINATED AGAINST PRRSV AND EXPOSED TO NATURAL INFECTION

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Introduction

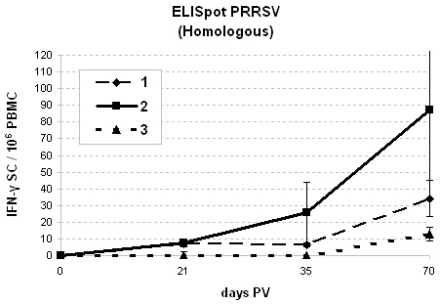
Previous studies have shown clinical protection induced by a modified live PRRSV vaccine when pigs were exposed to a heterologous field strain. Vaccine efficacy was associated with an efficient cell-mediated immune response. The ability of each isolate to induce a strong cell-mediated immune response might be more important than the genetic similarity between the vaccine and the field strains for inducing clinical protection [1, 2]. The present study aims at evaluating the IFN-γ SC response in vaccinated and naturally infected pigs after *in vitro* viral recall by using homologous and heterologous PRRSV isolates.

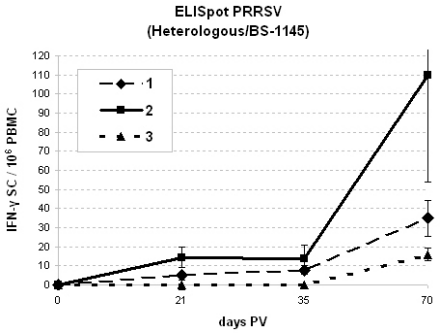
Material & Methods

Twenty-one PRRSV naïve pigs at 4 weeks of age were randomly assigned to three different groups. The pigs of group 1 (*n* = 7 pigs) and group 2 (*n* = 7 pigs) were vaccinated with Porcilis® PRRS at a dose of $10^{4.5}$ $TCID_{50}$ via the intramuscular (2 ml) and the intradermal routes (0.2 ml) respectively. Intradermal vaccination was performed using the I.D.A.L.® vaccinator. Pigs from group 3 (*n* = 7 pigs) received the adjuvant (Diluvac forte ®) only and served as controls. During the post-vaccination period (PV) the animals were kept in an isolation barn (0-35 days). Pigs were then moved to a conventional PRRSV-positive herd and naturally exposed to the resident virus. Blood samples were collected at day 0 (day of vaccination), 21, 35 and at day 70 PV (=35 days post-exposure) for the detection of specific antibodies by ELISA (Idexx) and for specific IFN-γ Secreting Cells (SCs) measurement. Levels of IFN-γ SCs in PBMC of pigs were determined according to Martelli et al. (2009) [2]. For the *in vitro* recall response, a vaccine virus solution (1 multiplicy of infection) (MOI) and an Italian isolate (BS-114S) were used as homologous and heterologous stimuli, respectively.

Results

The cell-mediated immune response evaluated by IFN-γ ELISpot assay showed a comparable trend for both routes of administration (IM and ID) after vaccination with low numbers of specific IFN-γ SCs up to 35 days. At day 70, control pigs were seropositive to PRRSV as a consequence of natural infection occurring in all groups (data not shown). At this time point the number of IFN-γ SCs showed a marked increase in vaccinated pigs, independent of the stimulus (homologous or heterologous) used in the *in vitro* recall (Figure 1).





Discussion & Conclusions

Vaccination stimulates a primary activation of the cellular compartment characterized by a low individual responsiveness in accordance with previous report [2]. After infection with a field virus the response in vaccinated pigs increases significantly with a higher number of SCs in ID-vaccinated animals as a consequence of a higher number of responders. The results obtained using different recall stimuli indicates that the amount of the IFN-γ SC response is more linked to the previous immune priming rather than the degree of genetic divergence.

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CONTROL OF A NEW ASIAN HIGHLY VIRULENT PRRSV STRAIN THROUGH VACCINATION. RECENT EXPERIENCE UNDER EXPERIMENTAL CONDITIONS

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Introduction

Since the first descriptions of PRRS outbreaks caused by highly pathogenic strains of PRRSV (HP-PRRSV) in China[1] to date, the disease has spread to other Asian countries. Both, the atypical genetic composition of some viral genes, and the greater pathogenicity exhibited by these emerging viruses have raise the concern in many veterinary practitioners about the effectiveness of current vaccines to control the infection, particularly in terms of productive performance. Here we describe a trial in which vaccinated and unvaccinated pigs were challenged with a HP-PRRSV strain.

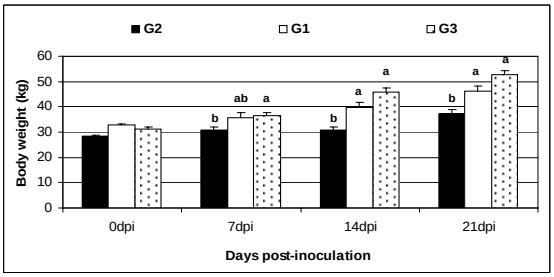
Material & Methods

Twenty-one 4-week-old, PRRSV-naïve pigs were randomly divided into three groups (G1 n=8, G2 n=8 and G3 n=5) and housed separately in isolation facilities (BSL3 at CReSA, Barcelona, Spain). Animals in G1 received intramuscularly (day 0) 2 ml ($10^{4.6}$ $TCID_{50}$/ml) of a European-type modified live PRRS vaccine (AMERVAC®PRRS). G2 and G3 pigs remained unvaccinated. At day +32, pigs in G1 and G2 were intranasally inoculated with 2 ml ($1x10^5$ $TCID_{50}$/ml) of the strain PRRSV21, which was originally isolated from a swine herd undergoing a severe PRRS outbreak in Asia. It shares over 99% similarity of the ORF5 nucleotide sequence with other HP-PRRSV strains, and belongs to the American genotype. Pigs in G3 remained uninfected along the trial. After challenge, weekly body weight (BW), average daily feed intake (ADFI) and clinical signs were assessed during 21 days post-inoculation (dpi). Also, blood samples were taken at days 0, 3, 7, 10, 14 and 21 pi. PCR in serum and ELISA serology were used to assess the response to vaccination and infection. Statistical analyses were done using a non-parametric Mann-Whitney test.

Results

Vaccination did not produce any adverse or side effect. After challenge, pigs in G2 suffered an evident respiratory distress starting on day 5 pi and lasting in most animals until the end of the trial. In G2, two animals died before the end of the study (25%). In G1 pigs showed milder respiratory signs and suffered no losses. All animals showed similar BW before the challenge. However, at day 7 pi G2 showed lower BW compared to G1 and G3 ($p<0.05$) and these differences remained statistically significant along the trial. Significant reduction in ADFI was recorded in G2 pigs from day 7 pi to the end of the study. ADFI differences were remarkably high at the end of the trial (G2=0.44; G1= 1.57 and G3= 1.97 kg/day/animal). Serology confirmed humoral responses after vaccination (G1) and subsequent infection (G1 and G2). Also, PCR confirmed infection (viremia) and viral clearance (G1 and G2).

Figure 1: Body weight of pigs vaccinated once with AMERVAC®PRRS and challenged with PRRS21 strain, compared with unvaccinated and PRRSV-naïve pigs.



a,b = $p < 0.05$; G1= vaccinated-challenged; G2=Non-vaccinated-challenged; G3= Non-vaccinated-non-challenged.

Discussion & Conclusions

PRRS-21 strain considerably affected the growth performance of non-vaccinated pigs (G2), which is a parameter that indicates high virulence traits in HP-PRRSV, as demonstrated in previous studies[1]. On the other hand, vaccinated pigs (G1) showed better growth performance and higher clinical protection. These results also suggest that nucleotidic sequence similarity among PRRS viruses is not a valid criteria to forecast cross-protection in the field, as demonstrated in previous studies[2].

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PHYLOGENETIC ANALYSIS OF CURRENT PRRSV STRAINS IN GERMANY

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Introduction

Sequencing of PRRSv-PCR positive samples has become a widely used procedure to gain more insights into the genetic background of currently circulating strains of EU and NA type in infected swine herds. Especially a clear discrimination between vaccine / vaccine mutant and wild type virus is desired. Here the authors provide a phylogenetic analysis of PRRSv sequences of routine diagnostic samples obtained at the *IVD GmbH* veterinary laboratory within the last five years.

Material & Methods

RNA of serum samples was extracted using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Germany). Tissue RNA was prepared with the RNeasy® Mini Kit (QIAGEN GmbH, Germany) as described by the manufacturer. Detection of PRRSv was performed by an orf7 based PRRSv-RT-nPCR discriminating between the European (EU) and North American (NA) genotype. Positive samples were subjected to full length orf5 sequencing. Aligned nucleotide sequences of orf5 were taken to establish phylogenetic trees by the neighbor-joining method using ClustalW and TreeView© software. The significance of the phylogenetic analysis was proven by 1000 bootstraps.

Results

Whereas EU-wildtype samples exhibited up to 15% sequence divergence to prototype strain Lelystad, sequences derived of the EU live vaccine strain *DV* and its mutants clustered closely to *Lelystad* virus. These EU vaccine strain mutants had up to 2 % divergence to a *DV* orf5 prototype sequence provided by the manufacturer and did show unique sequence characteristics separating them from *Lelystad* virus. Original prototype *Lelystad* sequences could not be found in the diagnostic field samples.

NA-type sequences had only up to 4% mutations with regard to reference strain *VR-2332* and are most likely descendants of a widely used American type PRRSv live vaccine. No NA-type wildtype strains other than the *VR-2332* phylogroup could be found in the field samples.

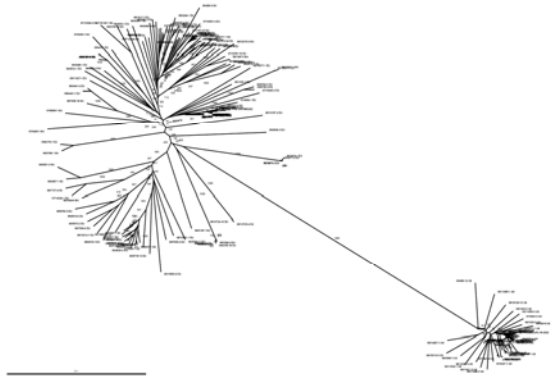

Fig. 1
Unrooted tree of PRRSv-EU and –NA sequences of routine diagnostic samples.

Discussion & Conclusions

PRRSv shows a tremendous amount of genetic versatility, constantly evolving new "strains" of quasi-species character. This poster provides an up-to-date overview of the current field situation of PRRSv in German pigs. The impressing genetic diversity of the EU wildtype samples still only reflects a fragment of the overall worldwide EU-type genetic spectrum as even more diverse isolates exist in Eastern Europe. In concordance with findings by *Cruijsen* et al. in the Netherlands Lelystad virus no longer exists in the field (1). Instead closely related strains of EU vaccine type *DV* can be found in herds with a vaccination history. As described earlier these mutants can be attributed to there *DV* origin by certain marker nucleotides that separate them from *Lelystad* virus (2).

Originally no NA-type PRRSv could be found in Europe. After the introduction of a NA-type live vaccine mutants of the *VR-2332* prototype strain appeared in swine herds. These seem to have started their own evolutionary development as they can be found independently of a preceding vaccination (3).

As up to now no virulence marker could be precisely determined in PRRSv nothing can be said about the clinical relevance of certain mutations either in wildtype or vaccine derived isolates, thereby limiting the benefit of sequencing for such purposes.

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ANALYSIS OF ELISA AND PCR TESTING FOR PRRSV IN GERMAN PIGS WITH REGARD TO MOTIVATION FOR EXAMINATION AND VACCINATION STATUS

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Introduction

Managing and evaluating diagnostic data is becoming increasingly important in order to get more information about animal diseases. Porcine reproductive and respiratory syndrome (PPRS) is one of the most important diseases in pig herds (1).This article analyzes the results of ELISA and PCR tests depending on vaccination status and reasons for examination.

Material & Methods

Between October 1, 2007 and September 30, 2009 27,077 porcine serum samples were analyzed serologically by IDEXX Herd Chek* PRRS 2XR Antibody ELISA and - as pools of 3-5 samples each - by nested PCR based on the ORF7 gene (2). For 17% of 27,077 sera and of 6,246 pool samples resp. the information about the causes for testing was known. Either the samples were part of a monitoring program with no actual health problems in the herd or reproductive problems, respiratory symptoms or other health problems in the herd were noted.

Results

Table 1 shows the number of tested pool samples and the percentage of positive samples with regard to motivation for testing and vaccination status of the herd.
Classified by motivation for examination about 74.5% of the 1350 samples from vaccinated herds were tested positive by ELISA with minor deviations depended on the reason for testing. In unvaccinated herds the variation is higher, the amount of positives by ELISA ranges from 37.0% (monitoring) up to 60.4% (respiratory symptoms). In total only 45.4% of the 1566 individual samples from unvaccinated herds were serologically positive (table 1).
There were a lower number of positive results in PCR. Over all 39.5% of the 294 pooled samples from vaccinated and 33.1% of the 347 pooled samples from unvaccinated herds were positive.

Discussion & Conclusions

Of the samples from vaccinated animals, 74.5% tested positive by ELISA whereas only 39.5% tested positive by PCR. This may be because of the shorter detection period for PRRSV after vaccination in blood than for antibodies. Even 37.0% of the unvaccinated animals without any acute health problems were serologically positive and in 26.1% of the pooled samples from "healthy" pigs PRRSV could be detected in the blood.
In non vaccinated herds (n_{ELISA}= 711 and n_{PCR}=115) there are similar amounts of positive results in monitoring samples and samples from herds with respiratory problems (ELISA positive: 48.9% and 42.6%,; PCR positive: 45.2% and 50.4%) whereas the amount of positive results in herds with reproductive symptoms is lower (8.4% tested positive by ELISA, and 4.3% tested positive by PCR). These data may indicate that PRRSV causes mainly respiratory problems.
As these data are derived not from randomized samples but from field samples and as the number of examined animals from unvaccinated herds with reproduction problems are lower than in the other groups these findings need further investigation.

Table 1: Number n of tested and percentage % of positive samples from vaccinated and unvaccinated herds depending on the motivation for testing.

	vaccinated herds		unvaccinated herds	
	% ELISA +	n 100%	% ELISA +	n 100%
Monitoring	74.9	833	37.0	941
Reproduction problems	73.9	257	48.8	123
Respiratory symptoms	73.8	260	60.4	502
sum	74.5	1350	45.4	1566

	% PCR +	n 100%	% PCR +	n 100%
Monitoring	49.1	169	26.1	199
Reproduction problems	13.4	67	16.1	31
Respiratory symptoms	41.4	58	49.6	117
sum	39,5	294	33,1	347

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AN OUTBREAK OF CLASSICAL SWINE FEVER IN ISRAEL

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Introduction

Classical Swine Fever (CSF) is a viral, multi-systemic, hemorrhagic and highly contagious disease, induced by a Pestivirus, RNA virus. CSF affects both reared and wild pigs, being the last considered as reservoir of CSF virus. Israeli swine population consists of 185,000 slaughtered pigs in 2008 (1) from 24 close-cycle units, of which 23 located in the Northern Region of Israel. The outbreak of CSF occurred in a close-cycle unit of 425 sows, with 6 boars and a total of 2000 fattening pigs (2)

Material & Methods:

Clinical pictures

First clinical signs appeared on 15/02/09 in one sow, with weakness, high temperature (> 41°C), skin redness. On 19/02/09 first abortions occurred; other sows showed anorexia, extended skin redness, limping, recumbence, difficulty to stand and move. Mortality reached 7 sows in 8 days. Death sows appeared cyanotic. At necropsy hemorrhagic lesions were present on parietal pleura, epicardium, diaphragm. Lungs presented interstitial pneumonia with hemorrhagic foci; in other cases, extended and severe fibrinous pleurisies, probably due to bacterial secondary infection. Spleen was increased in volume; lymph-nodes were congested and enlarged with hemorrhagic lesions at cutting surface; kidney presented typical multifocal cortical petechiae and multifocal hemorrhages in the cortex. Multifocal necrotic lesions were present in small intestine. Between 28/02/09 and 03/03/09, the Veterinarians of "Israel Nature and Park Authority" found 10 dead wild boars in a radius of 4 km from the herd. Laboratory investigations: have been finalized to confirm the suspect of CSF; to exclude African Swine Fever (ASF); Epidermitis Nephritis Syndrome (PDNS) from Porcine Circovirus type 2 (PCV2); acute pasteurellosis; *C. Novji* infection; intoxication from Aflatoxins, Citrinine, Ocratoxin. Porcine Reproductive and Reproductive Syndrome (PRRS) has not been investigated, being the Country negative. 24 sera samples from sows and 1 from wild boar have been submitted to antibody-ELISA test for CSF (PrioCHECK CSFV-Ab; Prionics; Lelystad, NL). Test reveals antibodies against E2 protein of CSF virus. Organs homogenates from 6 sows; 1 aborted fetus and 1 wild boar have been submitted to antigen-ELISA for CSF (PrioCheck CSF-Ag; Prionics; Lelystad, NL). Test reveals CSF virus in blood, plasma, serum, organs. Both ELISA tests have been executed at "Kimron Veterinary Institute", Bet Dagan (IL). Blood samples from 7 sows and 3 wild boars; organs homogenates from 6 sows, 1 fetus and 2 wild boars, have been submitted to RT-PCR towards CSFD virus at Virology Laboratory of Hannover (DE) Medicine Veterinary Faculty, OIE Reference Laboratory for CSF.

Results

12 out of 24 sows blood sera and the single wild boar sera sample resulted positive to antibody-ELISA test for CSF. 5 out of 6 organs homogenates from sows and the organs homogenates from fetuses and wild boar resulted positive to antigen-ELISA test for CSF. All the sera and organs homogenates from sows, aborted fetuses, wild boars, resulted positive to RT-PCR for CSF.

Discussion & Conclusions

Total losses were 103 sows (24%); 2 boars (33%); 355 fattening pigs (18%). Partial stamping policy out and vaccination with C-strain live vaccine were implemented in the Area. This is the first outbreak of CSF in Israel and in the Middle East in general.

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ENCEPHALOMYOCARDITIS VIRUS IN WILD RODENTS IN SWEDEN

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Introduction

Encephalomyocarditis virus (EMCV), a cardiovirus within the Picornaviridae family, has been reported to cause outbreaks of high mortality due to myocarditis in piglets and reproductive failure. The virus is world wide spread, and has the ability to infect many species of mammals, birds and insects. Rodents are acknowledged as the natural host, and between species transmission has been experimentally achieved (1). EMCV have never been isolated in Sweden, but a study on pigs from Swedish pig herds revealed 16.8% seropositivity, from which it was concluded that the infection is spread in the Swedish pig population (2). Considering the fact that wild rodents constitute the natural reservoir of the virus, its presence in the rodent population in Swedish pig herds was investigated, using a RT-PCR methodology for the direct detection of the virus.

Material & Methods

From eight pig herds, and six other, non-pig related locations (2 chicken farms, one mixed-farm and three urban locations), 125 rodents were caught in live or snap traps (table 1).

Table 1. Rodent samples

Type of location	Brown rat (*Rattus norvegicus)*	House mouse *(mus musculus)*	*(Apodemus flavicollis)*	
Pig farm	27	63	7	97
Chicken farm	0	13	0	13
Mixed farm	4	0	0	4
Urban location	9	2	0	11
Total	40	78	7	125

RNA was extracted from cardiac tissue with Qiagen RNeasy® Fibrous Tissue Mini Kit. For RT-PCR, QIAGEN® OneStep RT-PCR Kit was used, with primers CBA F and CBB R (3) for the amplification of the 5´-UTR region. The reaction started with reverse transcriptase step at 50°C, after which the PCR program continued with initial heating at 95°C for 15 min, followed by 35 cycles of 30s at 94°C, 1 min at 58°C, 1 min at 72°C, and a final extension for 10 min at 72°C. Positive amplicons of 602 bp were visualized by agarose gel electrophoresis and cleaned up with Wizard® SV Gel and PCR Clean-Up System. Sequencing was performed on 5 amplicons, using ABI PRISM BigDye Terminator V3.1. The sequencing was run on a capillary instrument, Genetic Analyzer 3100 (Applied Biosystems).

Results

By RT-PCR, 11 of 125 analyzed rodent samples were positive (8.8%). The proportion of positive samples was larger in pig herds (10.3%) than at other locations (3.6%). Positive rodents could be found in half of the examined farms, and one additional sample was found in an outdoor chicken farm. Sequencing of the amplicons showed high degree of similarity with EMCV sequences in gene bank and confirmed that EMCV RNA had been detected.

Table 2. Rodent samples positive by RT-PCR

Location	Type of location	House mouse (n)	Brown rat (n)	Proportion positive (%)
A	Pig farm	3		13.6
B	Pig farm	3		6.3
C	Pig farm	1		14.3
D	Pig farm	1	2	18.8
E	Chicken farm	1		20
		9	2	

Discussion & Conclusions

EMCV was detected by RT-PCR in mice and brown rats in Sweden. The results together with previous serological studies in pigs indicate that EMCV is a common infection on pig farms which should be considered a differential diagnosis in cases of high mortality and reproductive problems.

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ESTIMATION OF SENSITIVITY AND SPECIFICITY OF TWO SEROLOGICAL TESTS FOR HEPATITIS E ANTIBODIES IN PIGS

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Introduction

Hepatitis E virus (HEV) is divided into four genotypes and so far, only one serotype has been described. In endemic regions, serotypes 1 and 2 are mainly found in human population and genotype 3 and 4 in animals. In non-endemic regions, such as Europe, USA and Japan the situation is different and genotype 3 is found in both human autochthonous cases and animals (swine, wild boar or deer). HEV serological diagnosis is based on recombinant antigens or peptides derived from genotype 1 and 2. Considering HEV genotype 3 diversity (10 subtypes) [1] and quasi-species it is possible that serotype divergence might impair accurate serodiagnosis during genotype 3 infections. Furthermore, since swine is considered as a possible reservoir for human infection, it might be necessary to develop a serological test specific of swine strains. In this study, the performances of an ELISA test based on a recombinant baculovirus expressing a capsid protein of a swine genotype 3 HEV were compared to those of a commercial serological test adapted to swine but based on genotypes 1 & 2 using a Bayesian approach for correlated tests without gold standard.

Material & Methods

A preliminary validation of the VLPs based genotype 3 assay was performed using sera from experimentally HEV genotype 3 infected pigs and negative sera from SPF pigs. Results from these preliminary tests were used to define prior distributions for sensitivity and specificity. Further validation of the assay was performed using sera from 34 independent herds collected at slaughter house. All pig sera were tested using the VLPs based genotype 3 assay in comparison with the commercial serologic assays based on genotype 1 & 2 antigens. For HEV antibody testing no gold standard is available thus a latent-class Bayesian approach for correlated tests [2] was used to estimate the sensitivity and specificity of both tests. Prior distributions for prevalence, sensitivities, specificities were determined using Beta distributions because they are related to the Binomial distribution and give a good representation of this kind of biological data (unimodal distribution bounded within [0, 1] interval, possibly skewed). Parameters of Beta distributions were determined using external data: for prevalence, based on the available results from a current survey and for sensitivity and specificity, based on results obtained from a genotype 3 experimentally infected pig and in the SPF population respectively. Mildly informative priors were taken for prevalence at the individual level and sensitivity of both tests (they were assumed to be >0.6 with a mode at 0.9 with 95% certainty). More informative priors were taken for specificity as both tests gave negative results with samples taken from SPF animals, known to be negative for HEV. Thus specificities were assumed to be >0.95 with mode at 0.99 with 95% certainty. Analyses were carried out using Winbugs software.

Results

Similar apparent farm-level prevalence were observed using both test (74 and 77%) but higher prevalence within positive herds were observed using the genotype 3 VLPs based assay. The mean sensitivity of the commercial test was estimated to be 0.47 with 95% credibility interval being [0.39-0.55] whereas the mean sensitivity of the VLPs based genotype 3 assay was estimated to be 0.92 [0.81-0.99]. Specificities were estimated to be 0.98 [0.93-0.99] and 0.98 [0.95-0.99] for the VLPs-based and commercial tests respectively. The posterior estimate of the unbiased proportion of seropositive animals, given the tests' characteristics, was 0.49 [0.43-0.57].

Discussion & Conclusions

VLPs derived from swine HEV genotype 3 are good candidates for the improvement of hepatitis E serology in non endemic region and particularly into pigs. It is thus of major interest for a zoonotic agent with possible transmissions to human through food products.

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DETECTION OF *MYCOPLASMA HYOPNEUMONIAE* IN LIVE PIGS: COMPARISON OF FOUR SAMPLING METHODS

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Introduction

Mycoplasma hyopneumoniae (Mhp) is the primary aetiological agent of enzootic pneumonia in pigs and in association with bacteria and viruses is also involved in the Porcine Respiratory Disease Complex (PRDC) (1). The availability of accurate, rapid and easy to perform diagnostic tools is necessary for epidemiological and control purposes. The aim of the study was to assess the abilities of four sampling methods to detect Mhp by nested-PCR on live pigs in a field context.

Material & Methods

The study was carried out on a herd chronically affected by respiratory disorders. A sample of 60 pigs was constituted by a random selection from a batch of finishing pigs. Each pig was restrained and submitted to 4 samplings: oral-pharyngeal brushing, tracheo-bronchial swabbing, tracheo-bronchial washing and nasal swabbing. Mhp DNA was identified by modified nested-PCR (2). The sensitivity and specificity of each sampling method were estimated using a Bayesian analysis framework (3). Parameters distributions were based on previous external data. Since samples taken from SPF pigs gave negative results, a deterministic constraint was used for the specificities of all sampling methods which was taken as equal to one. The sensitivity of the parameter estimation to the choice of priors was assessed by comparing 3 models incorporating different sets of prior distributions ranging from vague priors (M1) to more informative ones (M3). Model convergence was assessed using the Raftery and Lewis tests and the Gelman-Rubin diagnosis. The models were compared on the basis of the deviance information criterion, the number of parameters estimated in the model and of the Bayesian p-value. The models were run using the freeware program WinBUGS.

Results

Mhp was detected by nasal swabbing, oral-pharyngeal brushes, tracheo-bronchial washing and tracheo-bronchial swabbing in 13.3%, 40.0%, 53.3% and 60.0% of the pigs respectively. Whatever the model, nasal swabbing had the lowest sensitivity and tracheo-bronchial swabbing the highest (Table 1).

Table 1: Mean and 95 % Credibility Interval of posterior distributions of the sensitivity of the four sampling methods of Mhp detection by nested-PCR, according to the 3 models with different prior distributions (60 pigs sampled, specificity=1 for all models and sampling methods)

Model*	Nasal Swabbing	Oro-pharyngeal Brushing	Tracheo-bronchial Washing	Tracheo- bronchial Swabbing
M1	0.19 (0.09-0.31)	0.51 (0.35-0.67)	0.66 (0.49-0.81)	0.72 (0.55-0.86)
M2	0.19 (0.09-0.32)	0.53 (0.38-0.68)	0.68 (0.53-0.82)	0.74 (0.59-0.86)
M3	0.25 (0.14-0.37)	0.53 (0.39-0.65)	0.62 (0.51-0.73)	0.73 (0.59-0.84)

*: M1: vague priors on sensitivities, specificity of the 4 sampling methods =1, M2: mildly informative priors on the prevalence, specificity of the 4 sampling methods=1, M3: mildly informative priors on the prevalence and parameters to estimate, specificity of the 4 sampling methods=1

Discussion & Conclusions

Since the infection status of the pigs tested under these conditions was unknown, and no gold standard is available, the sensitivities of the sampling methods were analyzed using a Bayesian approach. To the best of our knowledge, this is the first field study to use such an approach to evaluate four sampling methods for assessing Mhp infection in live pigs. The results of the present study indicate that tracheo-bronchial swabbing and washing, in combination with nested-PCR assay, were the most sensitive sampling methods for Mhp detection in naturally infected live pigs, the sensitivity of the tracheo-bronchial swabbing being slightly higher. As far as practical aspects are concerned, swabbing the tracheo-bronchial area with a sterile catheter is almost as convenient as obtaining nasal swabs under field conditions and only requires adding a gag to the sampling equipment. Tracheo-bronchial swabbing ensures a gain in diagnostic accuracy, being 3.5 times more sensitive than the nasal swabs commonly used in pig farms.

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COMPARISON OF DIFFERENT VACCINATION REGIMENS AGAINST *MYCOPLASMA HYOPNEUMONIAE* IN PIGS

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Introduction

This study was performed as a randomized, negative-controlled, blinded laboratory trial to compare the efficacy of a recently licensed 1-dose M hyo vaccine (Ingelvac MycoFLEX®, Boehringer Ingelheim) compared with that of two commercially available vaccines (1 and 2 dose products).

Material & Methods

Approximately 150 gestating gilts (all from a single breeding group week and previously M hyo-vaccinated) were blood sampled and screened on the Idexx HerdChek M hyo ELISA several weeks prior to farrowing. The 45 gilts with M hyo ELISA s/p ratios at or nearest to a s/p ratio of 0.70 (expected range of 0.40 to 1.0 s/p ratio) were identified and their piglets were included for the study. This range was used because it represents a typical sow herd M hyo serological distribution targeting the 50th to 75th percentile observed in the US swine industry.

Offspring were randomized at one week of age by sex using Excel. Pigs were weaned at 18 days of age and placed in growing pig facilities at the source farm/pre-challenge site until moved to the challenge site. Pigs were pre-medicated with tiamulin at the source/pre-challenge facility immediately prior to shipment to the challenge facility. The treatment group size (n = 50 per group) was derived from a power calculation using data from previous M hyo challenge studies and commercially available statistical software (Minitab®14 for Windows®). A summary of the groups and treatments are shown in Table 1. All pigs were necropsied 28 days post-challenge and lung lesions scored using the standardized PigMON® protocol by two evaluators. In order to determine differences among treatment groups concerning the observed lung lesion scores multiple comparisons were conducted using pairwise Wilcoxon rank sum tests followed by a Bonferroni-Holm adjustment of the p-values to keep the overall significance level of 0.05.

Table 1: Study design

Group	Vaccine	Vaccination Regimen	Challenge (pig age)
1	NVC	NA	9 wks
2	RESPISURE® ONE (1 dose)	2 ml IM at 3 wks age	9 wks
3	Ingelvac MycoFLEX® (1 dose)	1 ml IM at 3 wks age	9 wks
4	RESPISURE® (2 dose)	2 ml IM at 7 days & 3 wks age	9 wks
5	NVNC	NA	NA

Results

Non-vaccinated challenged (NVC) pigs had significantly higher lung lesion scores than nonvaccinated non-challenged (NVNC) pigs validating the M hyo challenge model. RESPISURE® ONE scores were not significantly different from NVC scores. Ingelvac MycoFLEX® and RESPISURE® (2 dose) scores were significantly lower than NVC scores. NVNC scores were lower than all other groups (Table 2).

Table 2: Lung lesion scores

Group	Vaccination	Mean % Lung Lesion Scores	Median % Lung Lesion Scores	Median Comparison[+]
1	NVC	11,8	11,2	A
2	RESPISURE® ONE (1 dose)	9,2	5,6	AB
3	Ingelvac MycoFLEX® (1 dose)	6,7	3,5	BC
4	RESPISURE® (2 dose)	4,9	1,9	C
5	NVNC	0,8	0,3	D

[+] Represents statistical significance of $p \leq 0.05$ if letters are different.

Discussion & Conclusions

This is the first large scale laboratory M.hyo challenge study with high power. The results of this study provide sound evidence that Ingelvac MycoFLEX® provided significant protection against M hyo challenge and was at least as effective as other well recognized one or two dose commercial vaccines.

SPES GRID and *ACTINOBACILLUS PLEUROPNEUMONIAE* ERADICATION IN A PIG HERD

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Introduction

A. pleuropneumoniae (App) infection causes acute pleuropneumoniae with mortality and a chronic form of respiratory disease mainly characterized by poor performances. Both conditions cause economic losses. The Authors describe the results of SPES (slaughterhouse pleurisy evaluation system) application at the abattoir on batches of pigs before and after the application of an App eradication program.

Material & Methods

The App eradication program was applied in a 1100 three-site production sow herd located in Northern Italy. From 2005 to 2008, pleuropneumoniae due to App infection had a very important economic impact on herd productivity. App biovar 1 serotype 9 was repeatedly isolated from pneumonic lungs of diseased pigs and a very high seroprevalence to APP has been demonstrated. The eradication program was based on partial depopulation of sows and complete depopulation of animals younger than 10 months of age with the exception of suckling piglets. After depopulation, site one housed 450 sows, sucklers and some boars. Two intramuscular injections of Enrofloxacin (5 mg /kg bw) in sow and Tulathromycin (2.5 mg /kg bw) in piglets were applied at 5 and 10 days interval respectively. In addition all gilts, sows and boars were offered medicated feed with Fluorfenicol (10 mg/kg bw). These antibiotics were selected on the basis of MIC (minimal inhibitory concentration) of App field strains isolated in the herd. In 2009, after the application of the over mentioned eradication program, 204 blood samples were tested for App antibodies using the indirect ELISAs IDEXX and VETQUINOL and all cases of respiratory disease were submitted to bacteriological investigations.

Chronic pleuritis (CP) at slaughterhouse were evaluated applying the SPES method (1) in the period 2005-2009. A total of 400 lungs (100 pig per batch) before and after the application of the eradication program were scored. The SPES grid provides two outputs for every inspected batch: the SPES average value (sum of single pleural scores/number of scored lungs) and the APP index (APPI=batch frequency of dorso-caudal pleural lesions (scores 2, 3 and 4) * f [SPES value in lungs with dorso-caudal lesions (scored 2, 3 or 4)].

Results

In 2009, after the application of the eradication program neither App isolations from pneumonic lungs died from respiratory disease, nor serological positivity for App were observed. Lung scoring by SPES grid at the abattoir before the application of the eradication program showed a high prevalence of CP lesions associated to App infection (grade 2, 3 and 4). Conversely, after the eradication process chronic pleural lesions referred to as caused by App infection were absent (figure 1 and table 1).

Figure 1: Distribution of pleural scores of pig lungs belonging to the herd from 2005-2009.

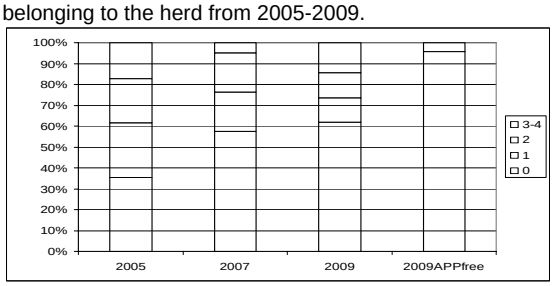

Table 1: S.P.E.S grid results and correlations among SPES/APPI values between batches evaluated from 2005 to 2009

Year	SPES media	APPIndex
2005	1,26[a]	1,02
2007	0,71[b]	0,52
2009	0,81[b]	0,68
2009 free	0,04[c]	0

[a,b,c]= Different superscript letters mean statistically significant differences ($p<0.01$)

Discussion & Conclusions

Twelve months after the application of the eradication program, clinical and laboratory findings demonstrates that the applied App eradication protocol had succeeded. SPES grid is a useful toll to be used in the monitoring of lung lesions at slaughterhouse and to follow up the measures of disease control or eradication at farm level.

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CYTOLOGICAL FINDINGS IN DIFFERENT PIG BREEDS DURING ACTINOBACILLUS PLEUROPNEUMONIAE INFECTION

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Introduction

The early innate immune responses during *Actinobacillus pleuropneumoniae* (*A.pp*.) infection are most decisive for the course and outcome of the disease. For this reason observed differences in disease susceptibility of Hampshire pigs (resistant) and Landrace pigs (susceptible) might be deduced to differing reactions of the innate immune system (1). Differential cell counts in blood and bronchoalveolar lavage fluid (BALF) have been evaluated to find differences in disease pathogenesis between different pig breeds. The influence of several pure-bleeding-lines on various blood parameters has been shown previously (2).

Material & Methods

116 weaning pigs from an *A.pp*.-free herd, 6-7 weeks old, of four different breeding lines (22 Hampsire, 25 Pietrain, 49 German Landrace and 14 Large-White) were infected experimentally in an aerosol chamber with *A.pp*.. Blood and bronchoalveolar lavage fluid (BALF) samples were taken prior to infection and either at day 4 or at day 21 after infection under deep anaesthesia with 15 mg/kg ketamine and 2 mg/kg azaperone. Blood was taken from the V. cava cranialis and BALF from the Bronchus trachealis using a flexible fiberoptic bronchoscope. BALF cytospots were stained with 10% May-Grünwald/Giemsa solution and 200 cells were differentiated for each differential count as it was done for blood differential cell counts

In parallel to manual BALF cell counting Combur® 9 test strips for urine analysis (Roche) were tested for their practical use for the estimation of BALF leucocytes.

Results

The highly susceptible German Landrace pigs differed from resistant Hampshire pigs showing less total cell counts in the blood at day 4 after infection, with a higher percentage of polymorphonuclear neutrophils and a lower percentage of juvenile neutrophils . At day 21 after infection Hampshire pigs showed a higher percentage of juvenile neutrophils in the blood. Prior to infection Hampshire pigs showed higher total cell counts in BALF which increased in the acute stage of inflammation. Semiquantitative BALF cell counts estimated by Combur® 9 test strips were positively correlated with total neutrophil and lymphocyte cell counts, but negatively correlated with the number of macrophages.

Discussion & Conclusions

Within different breeding lines clear differences have been observed in the severity of clinical disease symptoms and pathomorphological lung alterations, so that a genetically determined disease susceptibility against bacterial lung infections can be assumed (1). In this study the numbers of immune cells have been compared between the different breeds at different stages of infection. The observed cell counts in Hampshire pigs fit to the clinical picture: With high total cell counts in BALF this breed seems to be well prepared for local immune responses inside the respiratory tract to trap invading microorganisms. This might be an explanation for the differing systemic cellular responses in the blood in the acute stage of infection. Hampshire pigs showed a delayed shift to the left with juvenile neutrophils not before day 21 after infection (challenge).

In general a quick practical test for the assessment of BALF cell counts would be desirable. The semiquantitative leucocyte detection field on Combur® 9 test stripes is adjusted to human leucocyte enzymes. The field interpretation is not transferable to pigs and the sensitivity is not satisfying for the detection of leucocyte cell counts in porcine body fluids.

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SEROLOGICAL CHARACTERIZATION OF *HAEMOPHILUS PARASUIS* STRAINS IN ITALY

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Introduction

Haemophilus parasuis is the causative agent of porcine fibrinous polyserositis, arthritis and meningitis (Glässer's disease). *H. parasuis* is also commonly isolated from the upper respiratory tract. The information on the serovars distribution is essential in evaluating the possible benefits from vaccination (1).

Material & Methods

From 2007 to 2009 a total of 44 *Haemophilus parasuis* strains were isolated from diseased pigs in the diagnostics routine of Istituto Zooprofilattico Sperimentale of Lombardia and Emilia Romagna (IZSLER), Reggio Emilia Laboratory. The isolates were submitted to serotyping by agar gel immunodiffusion test (GD) by using specific antisera against serovars 2, 4, 5, 12, 13. The choice of the antisera used was performed considering the prevalence of different virulent serotypes described in other European Countries (1). In addition, the strains isolated in 2009 were serotyped combining the immunodiffusion test with the indirect haemoagglutination test (IHA) (2).

Results

In our study serovar 4 was the most prevalent (34%) followed by serovar 13 (22,7%) and serovar 5 (15,9%), while 22,7% of the isolates could not be assigned to a serovar (non-typable isolates). The strains could be divided into two groups depending on whether they were isolated from cases of systemic disease (polyserositis, arthritis or meningitis) or they only were isolated from the lower respiratory tract (table 1).

Table 1: *H.parasuis* serotypes prevalence and gross lesions observed.

Serotypes	%	Polyserositis	Bp
4 (15 strains)	34	11 (48%)	4 (19%)
13 (10 strains)	22,7	6 (26%)	4 (19%)
5 (7 strains)	15,9	3 (13%)	4 (19%)
12 (1 strain)	2,2	0	1
2 (1 strain)	2,2	1	0
Nt (10 strains)	22,7	2 (9%)	8 (38%)
Total		23	21

Nt: Non-typable; Bp: bronchopneumonia.

In 25 cases, five other species of swine bacterial agents were also isolated and identified: *Pasteurella multocida* (11/44), *Streptococcus suis* (5/44), *Escherichia coli* (4/44), *Actinobacillus pleuropneumoniae* (3/44) and *Bordetella bronchiseptica* (2/44).

Discussion & Conclusions

A percentage of 77,27 isolates has been serotyped by the GD test. The obtained results showed that the distribution of serovars in Italy is very similar to that recorded in other Countries (1). The comparison of the serovars distribution obtained from the respiratory tract of swine without polyserositis compared with those obtained from swine with polyserositis revealed a higher prevalence of serovar 4 in the latter case. The frequency of the isolation of serotypes 5 and 13 from pigs with or without polyserositis were similar, while non-typable isolates had a higher prevalence in respiratory disease compared to systemic infections. Although the serovars frequency and lesions observed are not statistically correlated (χ^2 test), our results are not in agreement with data reported by Angen et al. (2004). These conflicting results could be correlated with substantial genetic variability as well as strain differences in term of virulence within a serovar. Consequently, the definition of the serovar of an isolate cannot be considered as a reliable marker of virulence.

In this study *H. parasuis* was often isolated concomitantly with other bacteria. This should be considered when vaccination is applied for controlling disease outbreak and in the evaluation of the obtained results.

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THE ROLE OF DIFFERENT BACTERIA IN SOWS' MILK IN THE PATHOGENESIS OF PDS (POSTPARTUM DYSGALACTIA SYNDROME): MINOR OR MAJOR FACTORS?

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Introduction

Postpartum dysgalactia syndrome (PDS), with mastitis as main symptome, is an important disease in sows after farrowing associated with serious economic losses. These losses come along with the disease due to reduced performance of the sows and increased mortality of the piglets. Prevalence in herds can be as high as 60% [1]. As a multifactorial disease, PDS is influenced i.e. by management, feeding and hygiene, and, moreover, by bacterial pathogens. A wide bacteria spectrum has been isolated from the milk of diseased sows [2]. However, studies analysing the milk of healthy sows are rare. Therefore, this study aimed at pathogens which are possibly involved in PDS in the milk of diseased animals and compare this spectrum with the isolated bacteria in the milk of healthy control sows.

Material & Methods

Milk samples of 800 sows with PDS and 800 non-affected sows of different age were taken on four piglet rearing and fattening units. Sows were identified as diseased when the measured rectal temperature was above 39.5°C. Additional criteria like the clinical appearance of the mammary gland and the behavior of the piglets were considered. Moreover, pedigree information and possible risk factors were recorded and analysed. Bacteria involved in the pathogenesis of PDS were identified by advanced bacteriological analysis including molecular methods like PCR. The isolated *Escherichia coli* were analysed for various virulence genes with two multiplex PCRs [3].

Results

A wide spectrum of pathogens was isolated, belonging mainly to *Enterobacteriaceae*, *Staphylococcaceae*, *Streptococcaceae* and *Enterococcaceae. Escherichia coli* played the most important role with isolation rates over 70% in both PDS-affected and non-affected sows. No significant differences between diseased and healthy animals were detected in the occurrence of any bacteria species. With regard to the virulence genes, significant differences between strains from affected and non-affected sows were assessed for the virulence genes iron, kpsMTII, fimC and traT.

Discussion & Conclusions

Concerning the analysed bacteria spectrum, all species have been isolated in PDS-affected sows before [2]. Coliform bacteria were found more often than the other pathogens. In particular, *Escherichia coli* represented the major part of all isolated bacteria. These results are in accordance with several other investigations, showing that these pathogens probably play an important role in the etiology of PDS [2,4]. Two conclusions can be drawn from the fact that the bacteria spectrum in both healthy and diseased sows is similar: (a) virulence factors of bacteria species may contribute to pathogenesis; and (b) individual resistance of the single sow may change the susceptibility for PDS. Concerning the significant virulence factors detected in *Escherichia coli*, iroN and fimC were associated with urinary tract infections in humans [5]. *Escherichia coli* isolated from bovine mastitis possessed the gene traT [6], indicating a possible effect of this virulence factor. Further studies on bacterial virulence factors and genotyping of the sows with whole genome studies will follow this examination in order to deepen the understanding of the complex disease PDS.

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SUBCLINICAL URINARY TRACT INFECTIONS IN THE BELGIAN SOW POPULATION: A PREVALENCE STUDY

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Introduction and Objective

The prevalence of urinary tract infections (UTI) in sows in Belgium is not well known. In the neighbouring countries, the prevalence of (subclinical) UTI is estimated to be up to 20% (1). Urinary tract infections are responsible for sow mortality (2,3) and also have an impact on the breeding performance of the sow (1,4). Total born litter size, live born litter size and weaning litter size appeared to be lower in sows with UTI, while the wean-to-oestrus interval and the wean-to-farrowing interval increases in these sows. To investigate the prevalence of (subclinical) UTI in Belgium, a screening on Belgian sow herds was performed (project 'Veepeiler-varken' funded by the Sanitary Fund)

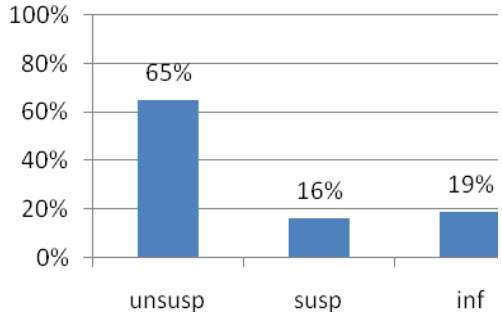
Materials and Methods

Twenty-five Belgian pig herds, with at least 100 sows and no clinical signs of UTI, were selected to participate in this screening. Drinking water supply was continuous in 11 farms, but it was restricted in 14 others. In each farm between 9 and 21 late-pregnant sows were sampled. A total of 353 urine samples was collected. Urine samples were collected in the morning, the first jets of urine were avoided. Bacteriological study of the urine was performed by inoculating 2 different media: Columbia sheep blood agar (Oxoid) and McConkey agar (Oxoid). Identification of grown colonies was performed after overnight incubation (37°C, 5% CO_2). Quantitative examination was performed through dilution of the urine samples in maximum recovery diluent (Oxoid) and subsequent inoculation of plate count agar (Biorad). Colony counting was performed after overnight incubation (37°C, aerobic). Nitrite detection was performed using the strip-test (Merckoquant; Merck).

Results

The predominant germs in the urine were *E. coli* (27%), *Streptococcus* sp. (15%) and *Staphylococcus* sp. (14%). In 38% of the cases no growth could be observed. In total, 19% of the sows showed urine bacterial counts higher than 10^5 CFU/ml, which should be interpreted as indicative of UTI. In 16% of the cases, an UTI could be suspected since the urine bacterial counts varied between 10^4 to 10^5 CFU/ml (Fig. 1). At herd level, 0 to 43% of the sampled sows suffered from a subclinical UTI. Comparing continuous or restricted drinking water access, a slight but non-significant difference in the prevalences of urine bacterial counts of higher than 10^5 CFU/ml could be observed , with 17% and 20% of urine samples of sows with continuous and restricted access, respectively, showing counts indicative for UTI. The strip-test, which is indicative for an *E. coli* infection, demonstrated 10% nitrite positive urine samples.

Figure.1 Distribution of urine samples according to the urine bacterial count. **Unsusp**ected (< 10^4 CFU/ml); **susp**ected (10^4-10^5 CFU/ml); **inf**ected (> 10^5 CFU/ml)



Discussion and Conclusions

The prevalence of subclinical UTI in the Belgian sow population, based on bacterial counts, is comparable to prevalence observed in neighbouring countries. Based on the strip-test, only half of the sows with a subclinical UTI can be detected. However, not all germs responsible for UTI have the capacity to produce nitrite. Essentially, *E. coli* can produce nitrite, whereas *Streptococcus* sp. and *Staphylococcus* sp., which can also provoke UTI, do not have a nitrite producing capacity.

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ARE SMALL RODENTS RESERVOIRS OF PATHOGENIC LEPTOSPIRA IN SWEDEN?

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Introduction

Leptospira species belongs to the phylum *Spirocheates* and the familiy *Leptospiracae.* The bacteria can be found all over the world and is accounted to be the worlds most widespread zoonosis. Under Swedish conditions leptospirosis can cause reproductive disorders in pigs with abortion and stillbirth (Swedberg & Eliasson-Selling, 2006). There are several diagnostic test developed for leptospirosis. Serology and culture are used in Sweden by Swedish Institute for Infectious Disease Control and Swedish National Veterinary Institute. Over the last decade the molecular method Polymeras Chain Reaction (PCR) has become an option. Rodents have been shown to be chronic carriers of *Leptospira* species (Levett, 2001) and the objectives of this study was to investigate whether small rodents captured in Swedish pig herds carry pathogenic *Leptospira* species. We chose PCR to analyse our samples.

Material & Methods

From seven pig herds, and four other, non-pig related locations (one mixed-farm, cow stable and two urban locations), 133 rodents were caught in live or snap traps (table 1).

Table 1. Rodent samples

Type of location	Brown rat	House mouse	Yellow-necked mouse	Water vole	
Pig farm	21	66	7	0	94
Mixed farm	5	1	0	0	6
Urban location	12	0	0	1	13
Total	38	67	7	1	113

Kidney tissue was aseptically removed and stored at -80^{o}C until analysis. DNA was extracted from the kidney tissue with DNeasy® Blood & Tissue Kit (QIAGEN Group, 2006) according to the instructions from the manufacturer. A conventional PCR method with the primers Adia214® and Adia215® was used (Branger et al., 2004) for amplification of the hap-1 gene. This gene can only be found in pathogenic *Leptospira* species. After agarose gel electrophoresis the amplicons were visualized with UV light. DNA purification of PCR products were done with a commercial kit, QIAquik ® PCR Purification Kit (50) (Qiagen). Sequences were processed with the CLC Main Workbench 5 and then compared against other deposited sequences in BLAST (http: / / www.ncbi.nih.gov / blast).

Results

PCR detected 15 samples of 113 as positive for pathogenic leptospira. The positive samples came from 11 house mice and two yellow-necked mice caught at four different pig farms, and one rat and one water vole caught at urban locations. Sequencing of the PCR-products confirmed that nine of the 15 DNA sequences belonged to pathogenic *Leptospira* spp. The nine DNA sequences showed 98-100% similarity to *L. borgpetersenii*, *L. weili* and *L. interrogans* serovar copenhageni (table 2).

Table 2. Result after sequencing

Type of location	Species	Result
Urban location	Water vole	100 % *L. borgpetersenii, L. weili*
Urban location	Brown rat	98 % *L. borgpetersenii*
Pig farm	House mouse	100 % *L. borgpetersenii, L. weili*
Pig farm	House mouse	96 % *L. borgpetersenii*
Pig farm	House mouse	100 % *L. interrogans* serovar copenhageni
Pig farm	House mouse	98 % *L. interrogans* serovar copenhageni
Pig farm	House mouse	100 % *L. interrogans* serovar copenhageni
Pig farm	House mouse	99 % *L. borgpetersenii, L. weili*
Pig farm	Yellow-necked mouse	99 % *L. borgpetersenii, L. weili*

Discussion & Conclusions

Pathogenic *Leptospira* spp. was detected by PCR in wild rodents in Sweden. Positive rodents could be found both in pig farms and at urban locations. All investigated rodent species in this study were positive, and could be considered a risk of spread of the bacteria to pigs, and presumably also to humans.

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FIELD TRIAL RESULTS USING DOXYCYCLIN (PULMODOX®) FOR ELIMINATING *LEPTOSPIRA BRATISLAVA* INFECTIONS FROM SOW HERDS

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Introduction

Leptospira infections of swine are spread worldwide. Because of their demanding growths characteristics the indirect serological testing by microagglutination technic (MAT) is broadly used. According Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of O.I.E. (Paris) a titer of ≥1:100 is significant for Leptospirosis (1). For therapy and prevention Tetracyclins are the commonly used. But the low resorption rates (2) of the less palatible classical Tetracyclins (CTC, OTC, TC) often lead to less tolerated dosages in feed of sows. Field experiences have shown that classical Tetracyclins resulted in not long lasting effects, although improvements in hygiene management to reduce reinfections from the enviroment were implementd. The main epidemiological reservoir for *Leptospira* are the kidneys of seropositve latent infected sows. In human Leptospirosis Doxycyclin is the first choice (4) because of its much better bioavailability after oral administration and its enhanced renal excretion (2, 3). Therefore Doxycyclin (Pulmodox®, Virbac) was tested to eliminate *Leptospira* from already infected sows.

Material & Methods

The trial were done in a new build farm unit during the first population phase. 330 in different stages pregnant gilts were stabled. A spot check blood sampling at the day of animal arrival had shown single animals (3/10) with MAT-titers ≥1:100. Three months later first clinical cases of Leptospirosis occurred. At this time which was the first day of the treatment interval (D0) all gilts (33/33) of a 10 % spot check (n=33) had already seroconverted for *L. bratislava* in the MAT. These animals were followed individually by repeated testing over a 12 months observation period (D21, D35, D50, D92, D166, D340). A successful elimination of *Leptospira* in each sow was proven by a declining MAT-titer and the final lost of antibodies (<1:100) over a long term period on herd level. All 330 sows were treated orally by 10 mg Doxycyclin per kg BW per day over a 14 days period (D0 to D14).

Results

There were no new cases of clinical Leptospirosis, no embryo- or maternotoxic effects and no teratogenity observed in Pulmodox® treated pregnant sows. The Doxycyclin treatment were well tolerated in all stages of pregnancy and all animals (n=330). The analyse of the percentages of the four MAT-titer classes demonstrated in table 1.

Table 1: Percentage of sows per MAT-titer class at different days of observation period.

MAT-titer	D0	D21	D35	D50	D92	D166	D340
<1:100	0	76	41	45	86	90	100
1:100 – 1:200	24	15	34	27	10	10	0
1:200 – 1:400	73	6	22	21	3	0	0
> 1:400	3	3	3	6	0	0	0
	100	100	100	100	100	100	100
Sows	n=33	n=33	n=32	n=33	n=29	n=30	n=10

Discussion & Conclusions

In summary it can be stated that the Doxycyclin treatment must have reduced *Leptospira* excretion efficiently. Together with the farm hygiene management reinfections could be prevented obviously. In contrast to some other publications the *Leptospira* specific antibodies are not persisting over a long period. In this trial a self-life of around 6 weeks could be demonstrated. Therefore the MAT could be used as a general control tool for the follow up of the redevelopment process. From the practitioner point of view a reciprocal correlation between the seroconversion status of a herd and its fertillity situation was shown by laboratoy data analysis in Germany.

The trial results demonstrated that it is possible to eliminate *Leptospira* infections from already infected sows by a Doxycyclin treatment. Together with a good working farm hygiene management a herd can redevelop from *Leptospira* infections.

To prevent the import of new *Leptospira* strains in healthy herds during introduction of external gilts, these gilts should be treated by Doxycyclin over a 14 day period at the end of quarantine phase before entering the reproductive herd.

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AN EXPERIMENTAL *HELICOBACTER SUIS* INFECTION REDUCES DAILY WEIGHT GAIN IN PIGS

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Introduction

Helicobacter suis (*H. suis*) is a Gram negative, long spiral shaped bacterium colonizing the stomach of more than 60% of pigs at slaughter age[3]. The prevalence is very low in sucklings, increases from the time of weaning and is highest in adult animals. This bacterium is considered to be one of the risk factors associated with gastric ulcers in pigs[2]. Recently *H. suis* has been successfully cultured in vitro[1].The purpose of this study was to evaluate the effect of an *H. suis* infection on the daily weight gain in pigs.

Material & Methods

In 5 different experimental set-ups, a total of 44 medicated early weaned piglets were intragastrically (n=39) or orally (n=5) inoculated once (n=30) or thrice (n=14) with a fresh (n=24) or frozen and thawed (n=20) culture of *H. suis* strain 5 at the age of 3 (n=5), 6 (n=14), 7 (n=10), 8 (n=5) and 9 (n=10) weeks. All animals were euthanized 4 (n=39) or 6 (n=5) weeks post-inoculation. All experimental set-ups included medicated early weaned animals as negative control groups (N=29) that were sham inoculated with sterile culture medium. The animals were fed a finely ground feed (n=34), coarsely ground feed (n=5) or milk replacement (n=5). All animals were housed under identical environmental conditions. Each animal was weighed at the time of inoculation and at euthanasia. Daily weight gain was calculated over the period after inoculation. In the combined results of the 5 experiments, the effect of inoculation with *H. suis* on the daily weight gain of inoculated animals was compared to the non-inoculated animals using a multivariable linear regression model including all variables that varied between the experiments as co-variables to correct for their effect (SPSS 17).

Results

After correcting for the effect of experimental set-up, type of feed, age at the time of inoculation, weight at the time of inoculation, number of inoculations, and the period after inoculation, there was a significant reduction of approximately 20 g/day (5%) in the daily weight gain of experimentally infected animals compared to the non-infected control animals.

Discussion & Conclusions

Results of the present study indicate that an *H. suis* infection reduces daily weight gain in pigs and thus may result in substantial economic losses. Clearance of infection with this bacterium may therefore have an economically beneficial effect.

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Acknowledgements

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RISK FACTORS FOR ACUTE DIARRHEA

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Introduction Identification of risk factors for diarrhea may lead to effective preventive strategies in the individual herd. The objective of the current study was to identify risk factors for diarrhea in outbreaks of acute treatment requiring diarrhea in batches of pigs between 10 and 70 days post weaning.

Material & Methods A case control study was conducted in 20 herds selected by multistage sampling in Denmark. Inclusion criteria were recurring therapeutic use of batch medication for diarrhea at room level in pigs between 10 and 70 days post weaning. Only intensive production systems were selected. One outbreak of acute diarrhea was investigated in each herd. The herds were visited the day following notification from the farmer or veterinarian of an acute treatment requiring outbreak of diarrhea. The farmer was not allowed to medicate before the pigs were examined. If the pigs had received antibiotic batch medication with-in the last 7 days of the examination day, the outbreak was excluded from the study. In each herd a sample of 80 pigs was selected by systematic random sampling among the pigs in the treatment requiring room. The selected pigs were subjected to a clinical examination. Among the examined pigs a simple random sample of 20 pigs with diarrhea and 20 pigs without diarrhea was selected and fecal dry matter (DM%) was determined. DM% ≤ 18.8 was considered as diarrhea (1) and used to classify the pigs as diarrheic (cases) or non-diarrheic (controls) in the statistical analysis. Unconditional screening of risk factors was performed using Chi-sq tests. A logistic analysis was performed using backward stepwise regression (selection criterion: p-value <0.05). Herd and pen were included as random effects and risk factors as fixed effects. Confounding effects and interactions were considered. All analyzes were performed in STATA IC version 11.

Results of the statistical analysis are presented in table 1.

Discussion & Conclusions The current study identified smaller size of a pig compared to penmates, long hair coat and having a lumbar cavity as risk factors for diarrhea in the individual pig. All 3 risk factors could either result from or predispose to development of diarrhea. It seems most likely that the 3 risk factors predisposed the pigs to diarrhea because only acute cases of diarrhea were investigated in the current study. Sorting batches of pigs by size into different pens at weaning is common in Denmark. The variable "average size" at pen level was not associated with diarrhea and no interaction with "size" compared to penmates was observed. This implies that the effect of being small compared to penmates applies for pigs whether or not a pen contains small, medium or large pigs compared to the average size in the batch. Interaction between "long hair coat" and "lumbar cavity" was observed. Pigs with both risk factors had lower odds of having diarrhea than pigs without both risk factors. This may be explained by long term anorexia in pigs with both risk factors.

Table 1. Risk factors for diarrhea (DM% ≤ 18.8) in a case (n=399) control (n=374) study of diarrhea

Risk factor	Categories	OR[#]	P-value*
Access to straw	Yes/No	0.96	0.78
Average size[1]	Small	0.95	0.78
	Medium	1	
	Large	0.84	
Mildly emaciated	Yes/No	0.9	0.69
Pot-bellied	Yes/No	1.73	0.31
Gender	Barrow	1	0.30
	Male	1.10	
	Female	0.85	
Pen hygiene	High	1	0.053
	Medium	1.51	
	Low	0.55	
Size[2]	Small	1.65	0.017
	Medium	1	
	Large	0.83	
Long hair coat (LHC)	Yes/No	1.88	0.014
Lumbar cavity	Yes/No	2.26	0.014
LHC + Lumbar cavity	Yes/No	0.13	0.005
Intercept = -0.09			
Random effect: $\sigma_{Herd} = 4.74*10^{-9}$			
Random effect: $\sigma_{pen} = 0.72$			

[1]Average size of pigs in pen compared to roommates,[2]Size of pig compared to penmates, [#]OR for dichotomous variables represents effect of the factor being present, *P-values represents overall significance.

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ESTIMATES OF THE BETWEEN PEN VARIATION IN OUTBREAKS OF ACUTE DIARRHEA

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Introduction

The major part of the antibiotic consumption in Danish pig production is used for treatment of gastrointestinal disorders in weaners. An increased knowledge of the clinical and infectious dynamics during outbreaks of acute diarrhea will improve preventive and treatment strategies and thereby decrease antibiotic consumption. The objective of the current study was to estimate the between pen variation of acute diarrhea in batches of pigs between 10 and 70 days post weaning

Material & Methods

A cross sectional study was conducted in 20 herds selected by multistage sampling. All herds serviced by six swine veterinarians from the same vet practice at Zealand and fulfilling the inclusion criteria were selected. The criteria were recurring therapeutic use of in-feed or in-water medication for diarrhea at room level in pigs between 10 and 70 days post weaning. Only modern intensive production systems were selected. One outbreak of acute diarrhea was investigated in each herd. All herds were visited the day following notification from the farmer/veterinarian of an acute treatment requiring outbreak of diarrhea. The farmer was not allowed to medicate before the pigs were examined. If the pigs had received antibiotic batch medication with-in the last 7 days of the examination day, the outbreak was excluded from the study.

A sample of 80 pigs in each herd was selected by systematic random sampling among all pigs in the nursery room where the outbreak of diarrhea occurred. Fecal consistency was evaluated for the selected pigs and assessed as normal, loose or watery. Loose and watery feces were combined into diarrhea in the statistical analysis. Feces classified as watery was also statistical analyzed separately as watery diarrhea. The observer's diagnostic sensitivity (Se) and specificity (Sp) for detection of diarrhea and watery diarrhea was calculated by comparing the clinical assessment to the "gold-standard" fecal dry matter (DM%) for a subset of the examined pigs (n = 773). DM% ≤ 18.8 was considered as diarrhea and DM% ≤ 13.1 was considered watery diarrhea (1). The data was analyzed using generalized linear mixed models with herd and pen as random effects. The *xtmelogit* command in STATA IC version 11 was used. The between pen variation was further evaluated by Intraclass Correlation Coefficients.

Results

The observer's diagnostic performance was Se = 0.88 and Sp = 0.91 for detection of diarrhea and Se = 0.64 and Sp = 0.98 for detection of watery diarrhea. A total of 1585 pigs representing 250 pens from outbreaks of diarrhea in 20 herds were included in the analysis. Results of the statistical models are presented in table 1. In an average herd (diarrhea prevalence = 0.36) the within pen prevalence of diarrhea was estimated to range from 0.18 to 0.60 for 95% of the pens. Variation between pens accounted for 6.7% of the total variation in occurrence of diarrhea. In an average herd (watery diarrhea prevalence = 0.11) the within pen prevalence of watery diarrhea was estimated to range from 0.05 to 0.23 for 95% of the pens. Variation between pens accounted for 5.4% of the total variation in occurrence of watery diarrhea.

Table 1. Result of generalized linear mixed models.

	Outcome variables	
Random effects	Loose and watery diarrhea	Watery diarrhea
σ_{Herd}	0.32	0.44
σ_{pen}	0.49	0.44
Intercept	-0.57	-2.06

Discussion & Conclusions

In nursery rooms with an outbreak of acute diarrhea variation between pens accounted for less than 7% of the total variation in occurrence of diarrhea/watery diarrhea. One explanation could be that non-infectious causes of diarrhea dominated in the selected herds. The result implies that the reason, for some pigs having diarrhea and others not within the same outbreak, is related to other characteristics than pens. The estimated 95% confidence intervals for within pen prevalence in average herds have implications for treatment strategies. Medication at room level rather than pen level will often be necessary since diarrhea will be equally prevalent in most pens at the same time during an outbreak in a nursery room.

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BRACHYSPIRA SPECIES DETECTED IN PORCINE FAECAL SPECIMENS IN SPAIN

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Introduction and Objectives

The bacterial genus *Brachyspira* consists of several species of intestinal spirochetes with the capability of colonizing a broad spectrum of hosts. *B. hyodysenteriae* and *B. pilosicoli* have been commonly detected in pigs suffering gastrointestinal disorders in Spain (1). However, there is no information about other species within the *Brachyspira* genus recovered from swine faeces. Accordingly, the purpose of the current study was to gain an overview of other *Brachyspira* species that colonize Spanish pigs.

Materials and Methods

Pure cultures of 67 Spanish field isolates of *Brachyspira* spp. from 54 farms were analysed. All isolates were weakly β-haemolytic, recovered from pigs between 2006-2009, and came from the collection held at the University of León. The isolates previously had been shown not to be *B. hyodysenteriae* or *B. pilosicoli* by the routine culture and PCR detection methods practiced in our laboratory (2, 3). A partial (~850 bp) sequence of the NADH oxidase (*nox*) gene was used for the identification of the 67 isolates. Sequence data for characterised isolates and reference strains retrieved from Genbank were included in the study, and are indicated in bold in the dendrogram. Sequencing analysis was performed according to previously described procedures (4). The genetic relationships between strains were visualised by constructing a dendrogram using the 'maximum composite likehood' model and the neighbour-joining tree in MEGA version 4 (5).

Results

Information from the partial *nox* sequences allocated most of the isolates to the three species *B. murdochii* (n=31, 46.3%), *B. innocens* (n=26, 38.9%) and *B. intermedia* (n=7, 10.4%). The three remaining isolates (4.4%) could not be included in any of the *Brachyspira* species that have been reported to naturally infect pigs. Identical isolates H29 and H51 were recovered from Iberian pigs belonging to 2 different herds. The *nox* sequence of these isolates had 63 nucleotide substitutions (7.4%) compared with the sequence of the *B. hyodysenteriae* isolates. The *nox* gene sequences showed greater heterogeneity amongst the *B. intermedia* isolates than amongst the other two species.

Discussion

Three species were identified amongst the presumed non-pathogenic *Brachyspira* isolates recovered from swine herds in Spain, of which

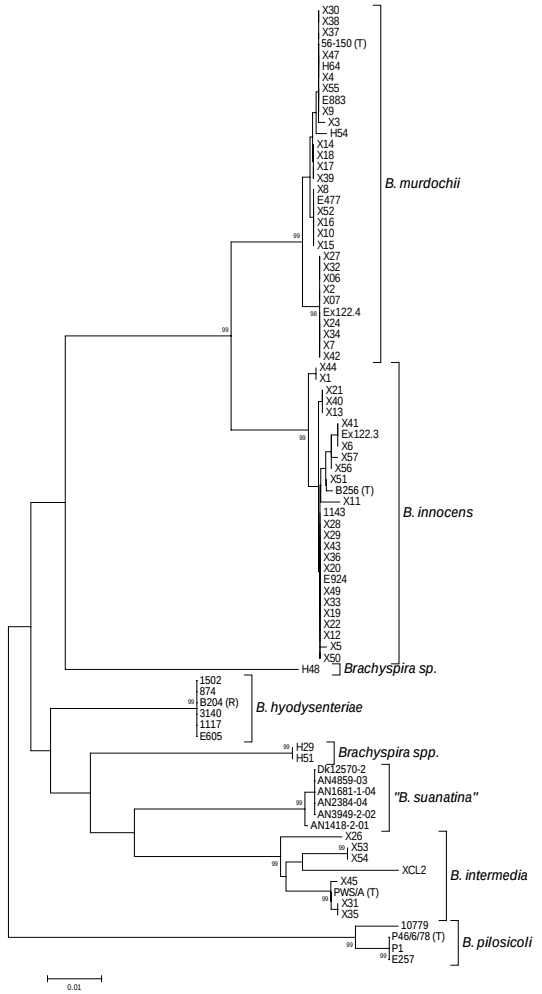

Fig. 1. Dendrogram based on partial *nox* DNA sequences from isolates belonging to all currently described *Brachyspira* species which colonize pigs.

B. murdochii and B. innocens were much more prevalent than *B. intermedia*. Although these species are generally regarded as being commensal in pigs, on occasion they all have been associated with mild colitis (6), and this is particularly so for *B. murdochii* (7). Three isolates could not be assigned to any of the *Brachyspira* species. Their clinical significance, distribution in other pig herds, and whether or not they may represent new species require further investigation. In conclusion, this study has added to knowledge about the biodiversity and evolution of *Brachyspira* species colonizing swine in Spain.

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GENETIC DIVERSITY AND POPULATION STRUCTURE OF *BRACHYSPIRA HYODYSENTERIAE* IN SPAIN

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Introduction and Objectives

The anaerobic intestinal spirochaete *Brachyspira hyodysenteriae* is the aetiological agents of swine dysentery (SD), a severe mucohaemorrhagic diarrhoeal disease that primarily affects pigs during the growing-finishing period. The research group at León University previously has found that more than 30% of Spanish farms and 12% of faecal specimens tested positive for *B. hyodysenteriae* (1). However, to date little is known about the genetic diversity and population structure of *B. hyodysenteriae* in Spain. The purpose of the current study was to use multilocus sequence typing (MLST) as a tool for investigating Spanish isolates of *B. hyodysenteriae* (2).

Materials and Methods

A total of 50 Spanish isolates of *B. hyodysenteriae* from 46 farms spread around the country, and one Portuguese isolate were analysed. All isolates were recovered from pigs affected with SD between 2001-2007 and came from the collection held at the University of León. Isolates were obtained from the most important pig production regions of Spain. Data for reference strain B204 were obtained from PubMLST and included in the study. Seven MLST loci were used, as previously described (2), and the MLST results were visualised by constructing a dendrogram using the unweighted pair-group method for arithmetic means (UPGMA) in START2 (3).

Results

The isolates were allocated to 9 sequence types (STs), of which 7 were newly described, in three major groups of descent (Fig. 1). Allele frequency ranged from 2 to 8 per locus. The predominant ST1 included 21 Spanish isolates from 18 farms in 8 different autonomous regions (Fig. 2). STs 2, 8 and 10 included several isolates from different regions. ST8 was widespread, while ST2 only appeared in the northern regions. Four singletons were identified (STs 4, 5, 6 and 9), including one represented by the Portuguese isolate H32. The isolates were moderately heterogeneous, as previously described for Australian isolates (2). Based on the number of isolates the population had an Index of Association (I_A) value of 0.786 and 0.990 based on the number of STs. Significant linkage disequilibrium was found in both analyses (P=0.001). For 5 Spanish farms where multiple isolates were available, 2 had isolates belonging to different STs (1 and 8). BURST analysis (not shown) indicated that ST1 and ST8 did not belong to the same clonal complex, demonstrating that genetically distinct isolates were present on the same farm.

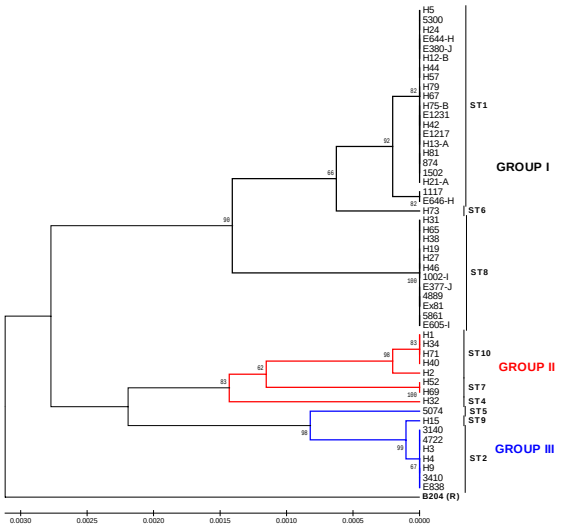

Fig. 1. Dendrogram showing the relationships of 9 sequence types (STs) obtained from 51 *B. hyodysenteriae* isolates. Reference strain B204 was also included (http://pubmlst.org).

Fig. 2. Map representing the autonomous regions of Spain. Regions where farms were located are coloured and the number of isolates/STs are indicated.

Discussion

This study has shown that Spanish isolates of *B. hyodysenteriae* are reasonably diverse, being divided into 9 STs in three major groups of descent. The bacterial population was clonal, with evidence of likely transmision of strains between farms all around the country. Some farms were infected with more than one ST, making control more problematic. The study has added to understanding of the diversity and population structure of *B. hyodysenteriae* in Spain.

Acknowledgements

This study was an international collaboration between the University of León, Spain, and Murdoch University, Australia.

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GENETIC DIVERSITY OF *BRACHYSPIRA HYODYSENTERIAE* ISOLATES FROM THROUGHOUT THE WORLD

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Introduction and Objectives

Swine dysentery caused by the intestinal spirochaete *Brachyspira hyodysenteriae* occurs worldwide and has a major economic impact. Phylogenetic analysis of bacteria using the sequence of housekeeping genes has enabled their segregation into the established species, and has also identified the presence of different subgroups within these species (1). This study used an established multilocus sequence typing (MLST) scheme (2) as a tool for investigating the genetic diversity of *B. hyodysenteriae*.

Materials and Methods

Existing data from a population including 162 isolates of *B. hyodysenteriae* from Australia (n=82; 50.3%), Europe (n=70; 43.6%) and North America (n=10; 6.1%) were analysed. These strains spanned 3 decades and originated from Australia (n=82), Spain (n=50), Sweden (n=10), the USA (n=7), Canada (n=3), the UK (n=5), Germany (n=3), Portugal (n=1) and Belgium (n=1). Most isolates were recovered from commercial pigs (n=152; 93.8%) but 6 were from feral pigs, 2 from mallards, 1 from a rhea and 1 from a mouse. Seven MLST loci (2) were used and distance was calculated by the maximum composite likelihood method and used to construct an unrooted radiation tree according to the unweighted pair-group method for arithmetic means in MEGA version 4 (3). Branch lengths were proportional to genetic distance.

Results

The number of alleles per locus for the 7 loci studied ranged from 9 (*adh*) to 21 (*gdh*), with a mean of 16.9. This implies that the number of different allelic profiles that this scheme can resolve (between 9^7 and 21^7) is very high. Consequently, it is unlikely that unrelated isolates exhibit the same allelic profile by chance. Based on the number of isolates the population had an Index of Association (I_A) value of 0.992, whilst based on the number of STs the I_A was 0.165. Significant linkage disequilibrium was found in both analyses (P=0.001). The Spanish isolates formed three subgroups (4), and are marked in colour on the tree (Fig. 1). The maximum distance between the Spanish isolates involved a total of 21 nucleotide substitutions over 4071 bp of the sequenced gene fragments (0.5%). These 3 subgroups comprised 72.2% of the isolates from the studied population, with the rest of the STs being arrange in a stepwise fashion with increasing genetic distances between them. The 5 more genetically distinct STs (ST61-ST65) contained recent isolates from Western Australia.

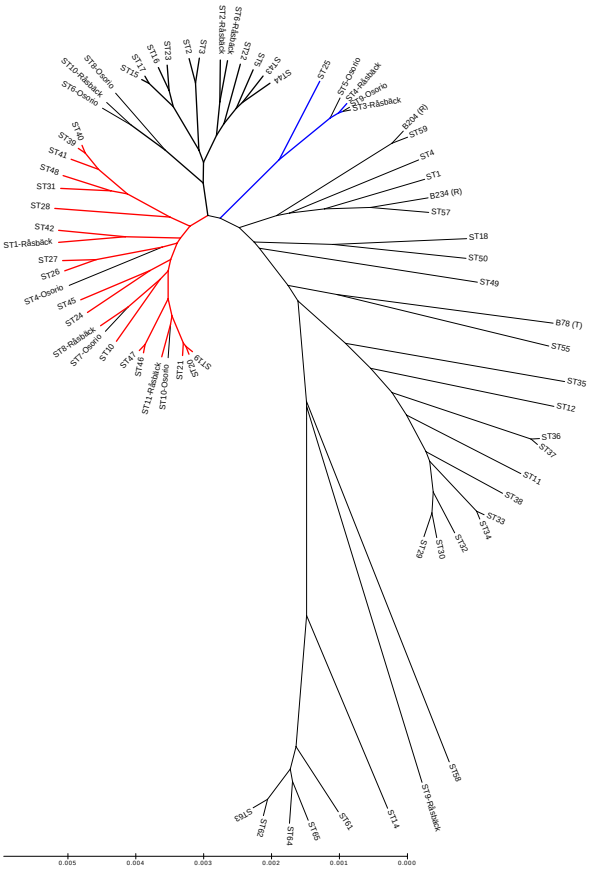

Fig. 1. Radiation tree showing the genetic relationships of 73 sequence types (STs) obtained from 162 *B. hyodysenteriae* isolates.

Discussion

The dendrogram showed as Fig. 1 confirmed previous observations that the specie is diverse (2). The Spanish *B. hyodysenteriae* isolates clustered in 3 differentiated lineages on the dendrogram, together with strains from different countries. This MLST scheme provided sufficient resolution power to unambiguously characterize *B. hyodysenteriae* isolates, and can be recommended as a routine typing tool that enables international comparisons of isolates. MLST analysis will generate data that will permit development of sophisticated models to explain the epidemiology and evolution of *B. hyodysenteriae* on a global scale.

Acknowledgements

This study was an international collaboration between the University of León, Spain, and Murdoch University, Australia.

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COMPARATIVE EFFICACY OF TWO RECOMBINANT PROTEIN-BASED VACCINES FOR SWINE DYSENTERY

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Introduction and Objectives

Swine dysentery (SD), caused by colonic infection with the spirochaete *Brachyspira hyodysenteriae*, remains a major problem worldwide. Despite this, there is no commercially available vaccine for SD. This deficiency is probably contributed to by a lack of knowledge about what constitutes a protective host immune response required for resolution of *B. hyodysenteriae* infection, combined with the lack of characterised protective antigens. SmpA (Bhlp16) is one of the outer membrane proteins (OMP) of *B. hyodysenteriae* that have been described (1). Vaccination with SmpB, an OMP of *B. hyodysenteriae* that shares the same locus as SmpA, has protected mice against a subsequent challenge (2). On the other hand, vaccination of pigs with recombinant His-tagged BmpB (Bhlp29.7), another OMP of *B. hyodysenteriae,* lowered the incidence of disease by approximately 50% following experimental challenge (3).

The purpose of the current study was to investigate the immunogenicity and protection against SD produced in pigs by vaccination with SmpA or BmpB.

Materials and Methods

The *smpA* gene was amplified from *B. hyodysenteriae* B204 using primers designed to engineer unique *Bam*H1 site into the final product. The amplified product and vector were digested with *Bam*H1 and ligated to produce pET28-SmpA. The plasmid was used to transform *E. coli* competent cells and expressed protein without a leader sequence was used. The BmpB protein was prepared as a His-tagged protein, as previously described (3). The reactivity of the recombinant proteins was evaluated by immunoblot using sera from pigs experimentally infected with *B. hyodysenteriae* B204.

Twenty-one 25-day-old conventional pigs from an SD-free farm were randomly assigned to three groups: group 1 (4 pigs) acted as unvaccinated controls; group 2 (8 pigs) were injected intramuscularly with 0.3 mg of recombinant SmpA protein emulsified in Freund´s incomplete adjuvant followed three weeks later by intragastrical administration of 0.5 mg of recombinant SmpA; group 3 (9 pigs) received BmpB following the same protocol as used for SmpA.

Two weeks after the second immunization the pigs were challenged intragastrically on 3 consecutive days with 10^{10} cells/day of *B. hyodysenteriae* B204. From then (PID 1) until the end of the experiment at PID 40, the physical characteristics of the faeces and the faecal excretion of *B. hyodysenteriae* assessed by culture and PCR were determined daily while blood samples were collected weekly. Serum antibody responses were measured in indirect ELISAs using recombinant SmpA or BmpB as plate-coating antigen, or using a whole-cell extract of *B. hyodysenteriae* (WC-ELISA).

Results

Both recombinant proteins were recognized by the B204 serum and gave clear bands at 16 kDa and 30kDa, respectively.

Vaccination with both proteins resulted in a good primary and secondary response, confirming the immunogenicity of the proteins. However, no specific response was detected after the vaccination using the WC-ELISA. A similar result was obtained by La *et al*. following vaccination with recombinant BmpB (3). On the other hand, the WC-ELISA clearly detected antibody responses in all animals as early as 2 weeks after the challenge.

Results of mortality (A), morbidity (B), duration of disease defined as days with diarrhoea (C), duration of bloody diarrhoea (D), duration of faecal excretion of *B. hyodysenteriae* (E) and incubation period (F) for the three groups of the study are showed in the table.

	Nr/Total (%)		Mean Nr. days (standard deviation)			
	A	B	C	D	E	F
Group 1 (control)	1/4 (25)	4/4 (100)	9.25 (5.8)	4.25 (4.3)	11.5 (5.8)	14.2 (9.0)
Group 2 (SmpA)	4/8 (50)	7/8 (87.5)	7.0 (4.9)	4.25 (3.1)	11.0 (8.6)	9.9 (5.0)
Group 3 (BmpB)	2/9 (22)	5/9 (55.6)	6.4 (2.3)	3.8 (0.8)	6.8 (3.7)	25.0 (4.7)

Discussion

To date, most evaluated vaccines against *B. hyodysenteriae* have only afforded partial, if any, protection from disease. Our results confirm that BmpB may partially contribute to protection of pigs against SD (3), while it is unlikely that SmpA confers protection. However, more experiments with larger numbers of pigs should be performed in order to confirm our findings. As previously proposed (3), complete protection might only be possible by vaccinating with a combination of proteins and cell components. In this regard, the recent availability of the genome sequence of *B. hyodysenteriae* has opened the door for new strategies of vaccine development for SD (4).

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MONITORING ANTIMICROBIAL RESISTANCE TRENDS IN SPANISH *Brachyspira hyodysenteriae* FIELD ISOLATES

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Introduction

Brachyspira hyodysenteriae is the causative agent of swine dysentery (SD), one of the most important gastrointestinal disorders among swine farms in Spain. Due to the lack of vaccines, control of SD generally involves the use of antimicrobials. In Spain, a recent study has investigated *in vitro* susceptibility of *B. hyodysenteriae* isolates from 2000 to 2007 to tiamulin, valnemulin, tylosin and lincomycin using a broth dilution technique (1).

The aim of the present study was to monitor susceptibility to tiamulin, valnemulin, tylosin and lincomycin in recent Spanish *B. hyodysenteriae* isolates.

Material & Methods

Bacterial strains and growth conditions: Fifty-one Spanish isolates of *B. hyodysenteriae* obtained from clinical submissions between 2008 and 2009 were investigated. All the isolates were from different pig farms distributed across the country. They were identified as *B. hyodysenteriae* according to their strong β-haemolysis and using a duplex PCR that also allows us to exclude *B. pilosicoli* mixed isolates (2).

Broth dilution procedure and antimicrobial agents: B. hyodysenteriae isolates were tested for antimicrobial susceptibility using VetMIC™ Brachy (ver.2) panels (SVA, Uppsala, Sweden) according to the manufacturer's protocol. The antimicrobial agents tested were tiamulin (TML), valnemulin (VNL), lincomycin (LNC), and tylosin (TYL). The minimum inhibitory concentration (MIC) was determined as the lowest concentration of antimicrobial agent that prevented visible growth. Absence of contamination was checked by phase contrast microscopy.

Statistical analysis: SPSS for windows was employed to perform survival analysis as previously described (1).

Results

The results of the susceptibility testing are shown in table 1. Log Rank test did not show statistically significant differences when TYL, LNC, TML and VNL survival curves from 2008-2009 were compared with the ones from previous years. A comparison of MIC distributions of tiamulin from 2008-2009 and 2006-2007, from a previous investigation (1), is showed in figure 1.

Discussion & Conclusions

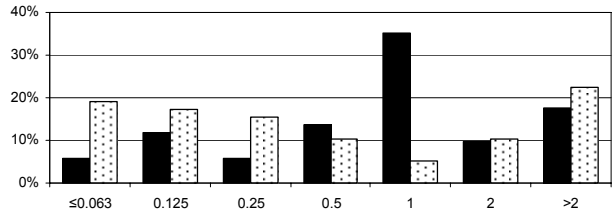
MIC_{50} and MIC_{90} for LNC and TYL were similar to those reported in Spanish *B. hyodysenteriae* isolates from 2006 to 2007 (1). However, a marked increase in MIC_{50} was recorded for TML and VNL in recent isolates, although MIC_{90} were similar when both periods of time were compared.

Table 1: MICs (mg/l) of four antimicrobial agents for 51 Spanish field isolates of *B. hyodysenteriae* recovered in 2008-2009. Data from 2006-2007 reported in a previous investigation is also shown (1).

Year of isolation		2006-2007 (n = 58)	2008-2009 (n = 51)
Tiamulin	MIC_{50}	0.25	1
	MIC_{90}	>2	8
	Range	≤0.016->2	≤0.063->8
Valnemulin	MIC_{50}	0.25	1
	MIC_{90}	>2	2
	Range	≤0.016->2	≤0.031->4
Tylosin	MIC_{50}	>256	>128
	MIC_{90}	>256	>128
	Range	64->256	16->128
Lincomycin	MIC_{50}	32	16
	MIC_{90}	128	>64
	Range	2->128	4->64

Survival analysis did not detect significant differences in susceptibility patterns of Spanish *B. hyodysenteriae* field isolates for the main antimicrobials used in Spain for treating SD over the last years. However, the MIC distributions showed a decrease in their susceptibility to tiamulin (Figure 1) and valnemulin, in agreement with the MIC_{50} detected changes.

Fig. 1. Distribution of MICs (µg/ml) of tiamulin for Spanish field isolates of *B. hyodysenteriae* recovered in 2008-2009 (solid bars) and 2006-2007 (dotted bars).



This study highlights the importance of keeping monitoring programs to detect emerging trends in antimicrobial susceptibility of *B. hyodysenteriae* field isolates.

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SENSITIVITY OF *Brachyspira hyodysenteriae* FIELD ISOLATES TO GARLIC DERIVATIVES

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Introduction

Brachyspira hyodysenteriae is the causative agent of swine dysentery (SD), one of the most important gastrointestinal disorders in pigs. Treatment and control of SD involve mainly the use of antimicrobials as no commercial vaccines are available. However, field isolates of *B. hyodysenteriae* with decreased susceptibility to one or more of the drugs commonly used for the treatment of SD have been reported (1, 2). These isolates represent a threat to the pig industry.

The antimicrobial properties of plants of genus *Allium* have been known for ages. Accordingly, the effectiveness of some garlic substances (*Allium sativum*) against some human gastroenteric bacteria have been reported previously (3, 4). However, knowledge on antimicrobial activity of these substances against animal pathogens is scarce.

This study aims to evaluate the potential use of two *Allium* spp. derivatives, propyl propyl thiosulfinate (PTS) and propyl propyl thiosulfonate (PTSO), to assist in the treatment and control of SD.

Material & Methods

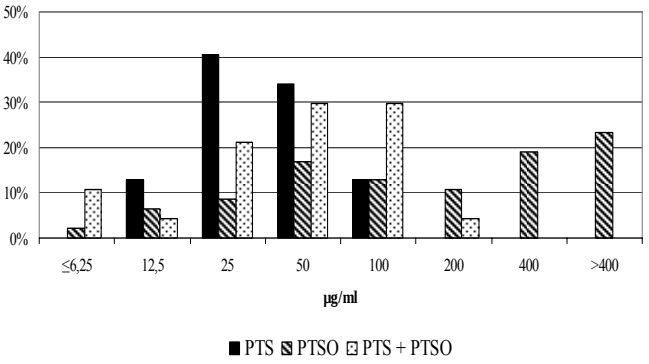
Bacterial strains and growth conditions. Forty seven isolates of *B. hyodysenteriae* from the bacterial collection held at the Animal Health Department at the University of León, Spain, were used in this study. Bacterial isolates had been recovered from different farms distributed across Spain between 2001 and 2009. Thawed isolates were grown on fastidious anaerobe agar supplemented with 5% horse blood in an anaerobic atmosphere (10% hydrogen, 10% carbon dioxide and 80% nitrogen) at 39ºC.

Table 1. MIC_{50} and MIC_{90} (µg/ml) of PTS, PTSO and PTS+PTSO for 47 Spanish field isolates of *B. hyodysenteriae.*

	PTS	PTSO	PTS+PTSO
MIC_{50}	25	200	50
MIC_{90}	100	>400	100
Range	12.5-100	≤6.25->400	≤6.25-200

Susceptibility panel and broth dilution procedure. A susceptibility testing panel was designed using twofold serial dilutions of PTS, PTSO and a combination of both called PTS+PTSO (17.5% PTS + 82.5% PTSO) [DMC Research Center SL, Granada, Spain] ranging from 6.25 to 400 µg/ml. For this purpose, 48-well plates were used, following the broth dilution method described by Karlsson et al. (1, 2). Growth control wells were included in each plate. MIC was determined as the lowest concentration of *Allium* spp. derivatives that prevented visible growth. Absence of contamination was checked by phase contrast microscopy.

Figure 1. Distribution of MIC of PTS, PTSO and PTS+PTSO for 47 Spanish field isolates of *B. hyodysenteriae.*



Results

The results and MIC distributions of the susceptibility test are shown in Table 1 and Figure 1, respectively.

Discussion & Conclusions

Although susceptibility of Spanish *B. hyodysenteriae* field isolates to PTS and PTSO were markedly different, MIC distributions indicated that growth of this bacterium was affected by the use of *Allium* spp. derivatives on their own or combined. PTS presented lower MIC_{50} and MIC_{90} than PTSO, showing a higher antimicrobial activity against this spirochaete. Accordingly, PTS was able to prevent the growth of all isolates at the tested range of concentrations, while PTSO enabled the growth of more than 20% of the isolates at 400 µg/ml. The combination of both substances retained the PTS antimicrobial effect in spite of decreasing its concentration to 17.5%.

The results of the *in vitro* susceptibility testing suggest that clinical trials should be performed to evaluate the *in vivo* activity of PTS and PTSO against *B. hyodysenteriae*. Control of SD could be improved by the use of *Allium* spp. derivatives.

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SPECIFICITY OF PCR TECHNIQUE COMPARED TO CULTURE FOR DETECTION OF BRACHYSPIRA HYODYSENTERIAE AND BRACHYSPIRA PILOSICOLI

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Introduction

Brachyspira hyodysenteriae, the etiological agent of swine dysentery, and less commonly *Brachyspira pilosicoli*, the infectious agent of spirochaetal diarrhoea, are frequent causes of colitis and diarrhoea in grower and finisher pigs. Especially swine dysentery causes considerable economic loss for pig farmers due to reduced weight gain, mortality, cost-intensive treatment, eradication and preventive measures. To get the disease under control, a quick and reliable laboratory diagnosis is a prerequisite. Up to date culture is a very common since highly sensitive method with the disadvantage of being extraordinarily time-consuming and therefore leading to delayed diagnosis with all its negative consequences. The use of PCR as a very rapid method could solve the problem, given a similar sensitivity and specificity to culture. However, veterinarians and farmers fear false positive results from PCR including all consequences for trading of piglets and gilts, and therefore sometimes prefer the cultural isolation. To evaluate the accuracy of PCR under field conditions, PCR applied in routine diagnostics was compared to culture for its level of agreement.

Material & Methods

Data obtained from routine diagnostics between Jan 2006 and Dec 2009 in North-Western Germany was analysed retrospectively: Results from faecal samples or anal swabs from pigs that were submitted to the Field Station for Epidemiology for the purpose of detecting enteric pathogens were extracted from a SQL database. Datasets were further filtered for those where samples had been examined for the presence of Brachyspira species by both methods PCR and culture. The multiplex PCR assay applied to the samples detects specific genome fragments of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* and additionally fragments of Lawsonia intracellularis (Nathues et al. 2007). For cultural testing, identical samples were examined on Brachyspira selective- and Columbia Blood Agar incubated anaerobically at 42 degree Celsius for at least 6 days. Differentiation of Brachyspira species was based on bio-chemical reactions and nox-RFLP. For statistical analysis of the data SAS ®, version 9.1, was used to calculate the κ-value as measure of concordance.

Results

During four consecutive years (2006-2009), 6,228 samples were tested for the presence of specific genome fragments of *B. hyodysenteriae* and *B. pilosicoli* by PCR. Overall, 184 samples were tested by both PCR and culture since the veterinarians and/or farmers did not rely on PCR alone. In 61 samples, unidentifiable *Brachyspira spp*. or others than *hyodysenteriae / pilosicoli* were detected culturally. These cases were excluded from further analysis to maintain exact comparability. Of the remaining 123 samples, 44 were positive for B. hyodysenteriae in PCR compared to 55 in culture, resulting in detection rates of 35.8% (PCR) versus 44.7 % (culture). Twelve samples identified as positive culturally were negative in PCR whereas one sample negative in culture resulted positive in PCR. The level of agreement is 80.1 %. Only in one sample *B. pilosicoli* was found (detection rate of 0.8 %), this being positive in PCR and culture, resulting in an agreement of 100%. The unweighted κ-value is 0.78, which is 96 % of the maximum possible κ-value of 0.82.

Discussion & Conclusions

This retrospective analysis on a subset of data obtained by routine diagnostic indicates a substantial to almost perfect agreement between results from PCR and culture to detect *B. hyodysenteriae* and *B. pilosicoli*. As mentionned, culture was preferred in terms of suspicion of better specificity than PCR in 58 submissions comprising 123 samples. However, results show that specificity of both methods were equal. Contrary, the PCR is lacking sensitivity when compared to culture, which might be caused by low concentration of the target in some samples or by inhibition of the PCR. Nonetheless, the PCR is a suitable alternative to cultural methods with comparable specificity for the diagnosis of B. hyodysenteriae and B. pilosicoli, with the added advantage of more rapid results.

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L. INTRACELLULARIS, *B. HYODYSENTERIAE*, AND *B. PILOSICOLI* IN GERMAN PIG HERDS, SIMULTANEOUSLY DETECTED AND QUANTIFIED BY REALTIME MULTIPLEX PCR

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Introduction

Brachyspira (*B.*) *hyodysenteriae* and *B. pilosicoli* are important swine pathogens causing swine dysentery and porcine intestinal spirochaetosis, respectively. Because of difficulties in culturing *Brachyspira* species and due to the obligate intracellular parasitism of *L. intracellularis* diagnosis of these bacteria primarily relies on the detection of genomic sequences. There is a close relation between the faecal load of *B. hyodysenteriae* and *L. intracellularis* and the clinical outcome of the infection (1, 2). Small amounts of these bacteria can often be isolated from subclinical cases and the positive result of a conventional PCR is often misinterpreted and does not lead to the causal diagnosis. Thus, to provide information from a clinical point of view, it is advisable to detect *and* quantify the putative agent of disease. We have developed a multiplex real-time PCR (mrtPCR) assay for the simultaneous detection and quantitation of *L. intracellularis*, *B. hyodysenteriae* and *B. pilosicoli* from faecal samples. The mrtPCR was applied to study qualitative and quantitative distribution of the three agents in commercial herds from Germany, in order to gain information on prevalences, bacterial loads, and the occurrence of mixed infections.

Material & Methods

A total of 1176 individual faecal samples from 95 herds (including 33.000 sows, 64.000 piglets and 145.000 fattening pigs) were sent in by 48 practitioners in the course of a diagnostic study. All samples were accompanied by a detailed questionnaire. For DNA extraction 300 mg of faeces were thoroughly mixed and applied to a Quiagen extraction kit. Finally, DNA aliquots extracted from 12.5 mg faeces were used for mrtPCR. Primers and probes for real-time PCR were designed with the Primer Express software (Applied Biosystems) targeting the *nox* gene of *Brachyspira* and the *aspA* gene of *L. intracellularis*. Gene sequences were retrieved from the Genbank database. Real-time PCR was performed on a 7300 Real Time PCR System (Applied Biosystems, Germany). The limit of detection was below 1 x 10^3 cells/g of faeces for all three species.

Results

From the individual samples, 12.7, 8.3, and 3.2 percent were positive for *L. intracellularis*, *B. hyodysenteriae* and *B. pilosicoli*, respectively. The relation of mono- to mixed infections per sample was 70.2%/29.8%. Regarding herds, 48.4, 24.2, and 31.6% were positive with *L. intracellularis*, *B. hyodysenteriae*, and *B. pilosicoli*, respectively. 68.4% of the herds were infected with at least two of the three organisms. 60.1%, 82.6%, and 73.3% of infections with *L. intracellularis*, *B. hyodysenteriae*, and *B. pilosicoli,* respectively, were mixed infections. Medians for the log number of cells/g of faeces (with 5% and 95% percentiles in brackets) were 3.3 (2.2-7.2), 5.9 (2.9-7.5), and 3.2 (2.1-5.8) for *L. intracellularis*, *B. hyodysenteriae*, and *B. pilosicoli*, respectively. Incidence and severity of diarrhea were correlated with the quantity of *B. hyodysenteriae* and *B. pilosicoli*, but not with that of *L. intracellularis*. Haemorrhagic diarrhea was associated with the presence of *B. hyodysenteriae* and was most severe with mixed infections of the two *Brachyspira* species. Of the positive faecal samples, 60%, 60%, and 80% did not correspond with the tentative clinical diagnosis of ileitis, dysentery, and both diseases, respectively.

Discussion & Conclusions

In conclusion, the three economically important pathogens have been shown to have a wide distribution among herds. The overlapping clinical signs, the relatively high incidence of mixed infections and the occurrence of *B. pilosicoli* in herds can lead to significant misinterpretations in the field, underlining the impact of a fast and reliable molecular diagnosis. The introduced mrtPCR can be a relevant tool in the diagnosis and prognosis of infections with *L. intracellularis*, *B. hyodysenteriae*, and *B. pilosicoli*.

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CASE REPORT: THERAPEUTICAL EFFECT OF VETMULIN® PREMIX ON A CLINICALOUTBREAK OF *B. MURDOCHII* IN A FATTENING HERD IN BELGIUM

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Introduction

Spirochaetes belong taxonomically to the order *Spirochaetes*, which contains several genera such as *Brachyspira* and *Leptospira*. The intestinal *spirochaetes* are included in the genus *Brachyspira*, of which five are known to be present in pigs: *B. hyodysenteriae*, *B. pilosicoli*, *B. intermedia*, *B. murdochii* and *B. innocens*. Porcine diarrheal disease in fatteners is mainly caused by *B. hyodysenteriae* and *B. pilosicoli*. In Belgium, *B. hyodysenteriae* has been diagnosed in 16% of the fecal samples (n = 2264) analyzed through PCR (1). In bacteriological culture, *B. hyodysenteriae* was present in 26% of the analyzed fecal samples (2). Besides the major pathogenic *B. hyodysenteriae*, *B. murdochii* also occurs in 9% of the fecal samples analyzed through PCR and in 5% of the bacteriological cultures for *Brachyspira* species. *Brachyspira murdochii* is generally considered as a minor pathogen (3). The present case report focuses on clinical outbreak caused by *B. murdochii* in a fattening herd in Belgium.

Materials and Methods

A fattening herd of 600 fattening places went through a period of clinical diarrhea in the group of 60-70 kg. The piglets all originated from one single sow farm. Clinically speaking the diarrhea consisted of flat feces with a slimy aspect without bloody contents. All fattening pigs were strongly contaminated with fecal material. Fecal samples of clinically affected pigs were collected for diagnostic purposes. The samples were cultured through standard anaerobic bacteriological *Brachyspira* culture and colonies were phenotypically identified to the species level.

Results

Bacteriological culture of *Brachyspira* resulted in the identification of *B. murdochii* in pure culture. As no other relevant bacterial species could be isolated from the fecal samples, it was considered to be the agent causing clinical diarrhea in the fattening herd. Following the bacteriological diagnosis, antimicrobial therapy with tiamulin (Vetmulin® premix; Huvepharma) at a dose of 10 mg active substance per kg live weight was started. The antimicrobial was administered through the feed for at least 7 days. Following the therapy, clinical recovery and normal fecal consistency could be observed after 5 days of treatment with Vetmulin®. After the treatment, clinical signs disappeared in the treated group. However, identical clinical symptoms were observed in other untreated fattening herds with piglets originating from the very same farm. In these fattening herds, similar therapy was introduced and resulted in identical therapeutical effect on clinical symptoms.

Discussion

The present case report clearly demonstrates that although *B. murdochii* is generally considered as a minor pathogen without specific clinical symptoms, occasionally clinical outbreaks can occur. The absence of bloody contents in the feces could indicate that *B. hyodysenteriae* was not the etiological agent, although clinical signs of *B. hyodysenteriae* in Belgium are not always associated with bloody feces. The importance of rapid and accurate diagnostics in order to install an efficient therapeutic plan is herewith demonstrated. The good therapeutic response of *B. murdochii* to the administration of tiamulin preparations is in accordance with the available data on antimicrobial susceptibility (4,5). In conclusion, the present case report clearly shows that *B. murdochii* is not as apathogenic as generally accepted. A clear relation with other piglets originating from the same farm could be observed, which indicates a contamination during the suckling or post-weaning period.

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EPIDEMIOLOGICAL STUDY OF ENTEROPATHOGENS (ROTAVIRUS, *ESCHERICHIA COLI*, COCCIDIA, *BALANTIDIUM COLI*) IN SUCKLING PIGLETS WITH DIARRHEA

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Introduction

Diarrhea in suckling piglets is a complex problem and causes considerable economic loss to the pig production. Is produced by many enteropathogens: rotavirus, coronavirus, enterotoxigenic *Escherichia coli* (ETEC), *Clostridium perfringens* type C and coccidia. Fecal samples and spleen collected from suckling piglets with diarrhea were screened for the presence of rotavirus, *E. coli,* coccidia and *Balantidium coli* using microscopic, culture and ELISA assays. Balantidiosis is an antropozoonosis that affected both humans and pigs.

Material & Methods

This study was carried out 80 fecal samples and 21 specimens of spleen from piglets identified with diarrhea. Fecal samples were tested for presence rotavirus using a commercial kits ELISA. The specimens (spleen) were cultured on blood, Levine and Mc Conkey agar plates. Coccidia and *Balantidium coli* were identified by microscopically examining feces for oocysts and cysts after flotation concentration method.

Results

The etiological diagnosis and rate of isolation of enteric pathogen from suckling piglets are shown in the next table.

Prevalence of enteric pathogen from suckling piglets (no. 101)

Enteric pathogens isolated	No.	Percentage (%)
Rotavirus	21	20,7
Coccidia	19	18,8
Balantidium coli	7	6,9
Escherichia coli	10	9,9
Total with single infection	**57**	**56,4**
Rotavirus + Coccidia	9	8,9
Rotavirus + Coccidia + *Balantidium coli*	7	6,9
Rotavirus + Coccidia + *Escherichia coli*	3	2,9
Rotavirus + Coccidia + *Escherichia coli* + *Balantidium coli*	1	0,9
Total with multiple infection	**28**	**27,7**
Negative	16	15,8

Rotavirus was the most frequently detect entropathogen in combination with other agents or alone, followed by coccidia, *E. coli* and *Balantidium coli*. As it could see in the table, 56.4% from cases had a monofactorial etiology and 27.7% had multiple etiology in suckling piglets with diarrhea.

Discussion & Conclusions

In Romania there are only a few studies which reports enteropathogens frequency in herds of swine. Rotaviruses are important causes of diarrhea disease in neonates and the young of many species inclusive in humans. Morin et al. (1) reports diarrhea in piglets at 1-3 weeks of age (55.8%), since Wieler reports a very low level of rotavirus infection in suckling pigs in Germany. In our study rotavirus, the most prevalent agent in suckling piglets with diarrhea (40.5%), was detected in 20.7% as alone infection and 27.7% as multiple infection. The results of our study indicate that coccidia is the most important protozoal disease of piglets causes diarrhea (38.6%). This finding corresponds to those of an earlier investigation in Germany (3) and Japan (1). *Escherichia coli* was isolated in 9.9% from single infection from suckiling piglets with diarrhea and in 1.4% from multiple infection. Similar results were obtained by others which reported on 12.8% alone and combined 6.8%.

Balantidium is usually found as a harmless organism in pigs, but it can sometimes cause severe clinical and even fatal disease. There are no studies that have examined the prevalence of *Balantidium coli* in pigs in Romania. In Danish farms the prevalence of *B. coli* is 57% in suckling piglets. In the present study the prevalence was 6.9% alone and 14.8% from multiple infections. In this study we are not found any agents in 15.8% of the suckling piglets.

On the basis of your results we can say that diarrhea in piglets was produce by multiple pathogens (84.1%). Regarding of this all prevention methods and treatment must be correlating with diarrhea pathogens.

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PATHOGENS ASSOCIATED WITH THE PRE AND POST-WEANING PORCINE DIARRHEA IN CUBA.

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Introduction

Contributing to a more integral view of the diarrhea syndrome, the objective was the study of its differential diagnose in Cuban suckling and weaned piglets as it has been depressed in the last years (Cabrera and Garcia, 2009).

Material & Methods

Fecal samples from sucking (0-25 days, *n*=45, S) and weaned (26-46 d., *n*=45, W) diarrheic piglets selected in 6 piggeries were tested by bacteria culture, agglutination, PCR, ELISA and chromatographic immunoassays kits as well as general parasitological methods for Rotavirus A (RA), Coronaviruses (TGEV, PEDV), *Salmonella*, *E. coli* (*E.c.,* ETEC and VTEC), toxigenic *C. perfringens* (T. *C.p.*) and its toxins (t) (α, β and ε), *C. parvum* (*Cr.p.*), *Eimeriidae* (*I.suis*) and helminths.

Results

PEDV and helminths were not detected. The isolates STa,STb$^+$ and F4,STa,STb$^+$ were the more common ETEC. ETEC from S piglets were positive also for F5, F6 and F41 and in W piglets for F6 and LT. F18$^+$ ETEC and VTEC (VT2e$^+$) were only present in W piglets. The only diagnosed *Salmonella* sp. was *S. enterica* var. Newport in W piglets. (Fig 1, Table 1).

Discussion & Conclusions

Lazo et al., (2005) clinically reported a 43.8 % of morbidity and 29.3 % of lethality due to swine enteric colibacillosis in Villa Clara, Cuba. The present survey demonstrated that diarrhea in Cuban S and W piglets is polyetiologic, with a high variety of associations among pathogens that makes difficult the outbreaks management. The pathogens prevalence differs in S and W piglets and among piggeries. For improving the survival rate of S and W piglets, it is necessary to implement in practice a specific surveillance and control program for enteric disorders in every farm.

Fig 1. Prevalence (%) of diarrheagenic agents in S and W piglets

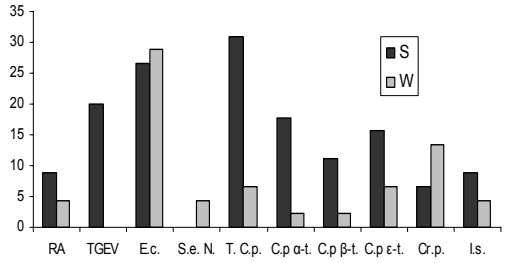

Table 1: Founded polyetiologic conditions.

Associations		n
TGEV/*C.p* β,ε-t	TGEV/*C.p* ε-t	2
Cr.p./C.p α-t	*Cr.p./E.c.*F4$^+$	1
Cr.p./C.p β,ε-t/*E.c.*STa,STb$^+$		1
Cr.p./S.e. Newport/*E.c.*F4,STa,STb$^+$		1
*Cr.p./I.s./E.c.*F4,STa,STb$^+$		1
I.s./C.p ε-t/*E.c.*STa,STb$^+$	*I.s./C.p* α-t	1
*I.s./E.c.*F4,STa,STb$^+$		1
I.s./RA/*E.c.*F4,STa,STb$^+$		1
RA/*E.c.*STa$^+$	RA/*E.c.*STa,STb$^+$	1
RA/*E.c.*F4,STa,STb$^+$		1
C.p ε-t /*E.c.*F18,VT$^+$		1
C.p α-t/*E.c.*F6,STa$^+$/*E.c.*Sta$^+$	*C.p*β,ε-t	1
C.p α,β,ε-t		2
*E.c.*F6,STa$^+$/*E.c.*F18,VT$^+$/*E.c.*Sta$^+$		1
*E.c.*F6,STa$^+$/*E.c.*F41,F5,Sta$^+$		1

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DISK PREDIFFUSION IS A RELIABLE METHOD FOR TESTING COLISTIN SUSCEPTIBILITY IN PORCINE *ESCHERICHIA COLI* STRAINS

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Introduction

During the last few years, acquired resistance to colistin in *Escherichia coli*, but also in other bacterial species, has been reported. It has been shown that the disk diffusion test is not a reliable method for the detection of this resistance. Therefore, there is a need for a reliable and cheap test to determine colistin susceptibility of pathogenic *E. coli* strains.

Material & Methods

In the current research, the colistin susceptibility of one hundred and fifty seven (157) *E. coli* strains isolated from independent clinically affected pigs during the period 2005-2006 was determined. Antimicrobial susceptibility testing was carried out using 4 different techniques. As golden standard, all strains were tested for susceptibility to colistin through the agar dilution (AD) method (CLSI, 2008). In addition the strains were also evaluated using the Kirby Bauer disk diffusion test (Neosensitabs, Rosco); 2 + 18 hours disk prediffusion test (Neosensitabs, Rosco) and E-test (AB Biodisk). Disk diffusion and disk prediffusion inhibition zones and MICs determined by the E-test were statistically analysed and compared with the MICs determined by the reference agar dilution assay for each strain.

Results

Using the results obtained by the AD method, a clear bimodal distribution of MIC values was observed (Table 1) with 15 strains (9.6 %) located in the non wild-type cluster of the bimodal distribution.

Table 1: MIC values of porcine *E. coli* strains

Test	Number of strains with colistin MIC values (µg/ml) of							
	0.25	0.5	1	2	4	8	16	32
E-test*	18	64	37	22	3	8	4	1
Agar dilution	4	117	20	1	1	11	3	

*E-test values were rounded up to the next highest doubling dilution
The clinical breakpoints for susceptibility (MIC ≤ 2 µg/ml) and resistance (MIC ≥ 8 µg/ml) that were used for all comparative analyses are represented by a discontinuous and a solid line respectively.

The results of the comparative analysis are summarized in Table 2. The disk diffusion results showed both a low categorical agreement and a low correlation with the results obtained by the AD method. In addition, the percentages of very major and minor errors exceeded the acceptable levels. The results obtained by E-test and disk prediffusion assay generated percentages of minor, major and very major errors beneath the acceptable levels. The categorical agreement and correlation with the results obtained by the AD method were very good for both tests.

Table 2: Discrepancy rates, categorical agreement, and correlation between AD MIC values and DD, PD and E-tests values

~Agar Dilution	Very major error < 1.5%§	Major error < 3 %§	Minor error < 10 %§	Categorical agreement§	Correlation coëfficient
DD	1.9 %	1.3 %	49.7 %	46.5 %	0.09
E-test*	0 %	0.6 %	1.9 %	96.8 %	0.64
PD	1.3 %	0.6 %	0.6 %	96.8 %	0.80

* E-test values were rounded up to the next highest doubling dilution
§ Breakpoints used: Disk diffusion (DD): resistant ≤ 16 mm; sensitive ≥ 20 mm; Disk prediffusion (PD): resistant ≤ 10 mm; sensitive ≥ 15 mm; Agar dilution and E-test: sensitive ≤ 2 µg/ml; resistant ≥ 8µg/ml

Discussion & Conclusions

The current results show that approximately 10 % of the investigated porcine *E. coli* strains belonged to the non wild-type population , indicating acquired resistance towards colistin. Until now, no clinical breakpoints for this antibiotic are available for (oral) veterinary use. The human CLSI breakpoints for colistin are for parenteral formulations (CLSI, 2009) and therefore may not predict clinical efficiency of oral formulations in animals. The present results suggest that the E-test and the 2 + 18 prediffusion test are reliable methods to test colistin susceptibility in porcine *E. coli* isolates, while the disk diffusion test is not. As many laboratories still rely on the disk diffusion test, the emergence of colistin resistance may be missed.

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INCREASED ANTIMICROBIAL RESISTANCE FOR COLISTIN OF HAEMOLYTIC *E. COLI* STRAINS FROM CLINICAL CASES IN BELGIUM USING THE E-TEST

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Introduction

The last few years, acquired resistance to colistin in *Escherichia coli* (*E. coli*) has been described quite frequently. It has been shown that the standard agar dilution test is not a reliable method for the detection of colistin resistance in several bacterial species. Therefore, the need for a reliable test to check colistin susceptibility of pathogenic *E. coli* strains in routine diagnostics was rather high. A practical study, comparing 4 different antimicrobial susceptibility tests revealed that the E-test was the most reliable alternative agar-based colistin susceptibility testing method for use in *E. coli* strains (1). The objective of the present study (project 'Veepeiler-varken', financed by the Sanitary Fund) is to evaluate the evolution of results obtained following the introduction of the E-test in the diagnostic laboratory procedure.

Materials and Methods

All samples from the content of the small intestine -positive for hemolytic *E. coli*- were tested using the E-test (AB Biodisk) with direct reading of the minimal inhibitory concentration (MIC) and expressed in µg/ml. The interpretation towards susceptibility or resistance is presentated in Table 1.

Table 1. Interpretation of antimicrobial susceptibility testing to colistin using the E-test

Interpretation	µg/ml
Susceptible	< 4
Intermediately susceptible	4-16
Resistant	> 16

Results

The distribution of antimicrobial susceptibility testing of the (haemolytic) *E. coli* strains isolated from diagnostic bacteriology of samples from small intestines in the period 2006-2009 are presented in Figure 1. Overall, an evolution from 8% intermediately susceptible strains to colistin in 2006 to 28% in 2009 could be observed. On average, 150 strains of haemolytic *E. coli* were tested in routine diagnostics yearly.

Discussion and conclusions

Colistin is used worldwide as an oral antimicrobial agent for the prevention and treatment of neonatal and weaning-associated *E. coli* infections in piglets.
Considering its extensive use and the fact that antimicrobial formulations are often underdosed (2), it is not surprising to find an increasing colistin resistance in veterinary *E. coli* strains (3).

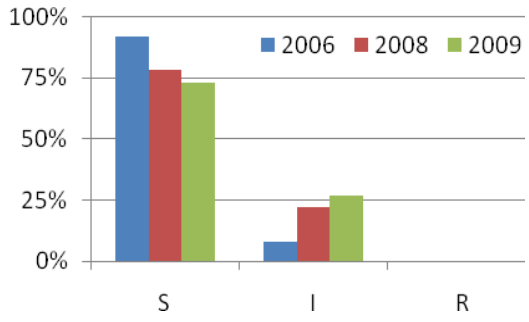

Figure 1. Distribution of antimicrobial susceptibility results with the E-test obtained from (haemolytic) *E. coli* strains isolated in diagnostic bacteriology from 2006 till 2009. S: susceptible, I: intermediate, R: resistant

Following introduction of a more reliable antimicrobial susceptibility testing method, using the E-test, the occurrence of a high percentage intermediately susceptible *E. coli* strains could be observed in Belgium. This is in accordance with the information provided by veterinary practitioners concerning the decreasing efficacy of colistin in the treatment and prevention of *E. coli* infections, especially after weaning. Monitoring of the further evolution of antimicrobial susceptibility will be necessary in order to guarantee treatment success when applying colistin in antimicrobial formulations for piglets post-weaning.
In conclusion, further monitoring of colistin resistance is advisable to omit excessive therapy failure in the near future.

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AN ETEC REDUCTION DERIVED BY SACCHAROMYCES CEREVISIAE ADDITION IN WEANED PIGLETS

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Introduction

An intestinal disorder of piglets before weaning causes loses either by direct mortality or by malabsorption and intestinal dysfunction. Disorders of milk intake and consecutive lack of lactogenic immunoglobulin supply enable grow of pathogenic microflora, ready to affect absorption and caused fatal dehydratation.

Significant positive results have been produced with probiotics (bacteria, yeasts) fed to the piglets and to the sows.(1) Enhanced numbers of favourable bacteria correlate to decreased numbers of potentially pathogenic together with more favourable profiles of fermentation products along the intestines. In contrast, measurable effects of these dietary factors on intestinal physiology and mucosal immunology have been limited or difficult to interpret in many cases. (2)

The influence of addition of live culture of *Saccharomyces cerevisiae* (Sc) to the feedstuff of sows and piglets before weaning is further studied.

Material & Methods

Four groups of 16 sows each were formed with average 11.8 piglets per litter. First group of sows and litter was fed by sows' feedstuff and piglets' prestarter premix, supplemented with Sc culture at 1000 ppm each (Actisaf Sc47, Lesaffre Feed Additives, France). In the second group the litters only were served by prestarter enriched with Sc, in the third group only sows were fed by the feedstuff enriched with Sc. The fourth control group was fed by feed without any Sc addition. The piglets were clinically monitored every week and health changes, especially depression, inappetence and scour were noted daily. The swabs from litters were collected in week intervals. The swabs were cultivated and haemolytic strains of *E.coli* were examined in respect of closer differentiation of the pathogenic factors. the litter was weighed after birth and before weaning. The obtained data were statistically evaluated by Kruskal-Wallis non-parametric test, performed by StatSoft software.

Results

The Sc dietary supplementation to sows and piglets reduced significantly number of ETEC colonies at 2nd and 3rd week about 8 and 14% ($p=0.030$ and 0.044) respectively, followed by lower or nil diarrhea symptoms and lower mortality, reduced mortality in Sc group of 1.03 piglet per litter ($p=0.04$) in compare to Control group. A tendency to reduction of mortality was find out as well in the groups where only the sows or piglets received *Sc.*

Discussion & Conclusions

The microbiological findings confirmed the major causative agent of diarrhea expectedly as *E.coli*. Piglets seem to not respond to addition of *Saccharomyces cerevisiae* into diet by lowering of appearance of either *Cl. perfringens* or *Isospora suis* in rectal swabs (data not shown) and the treatment seemed to have no measurable effect on the number of pigs, positive to both of infections.

On the contrary, the group served by feedstuff enriched with Sc to both, the sows and piglets, showed significant lower number of findings positive to hemolytic *E.coli* in compare to controls and also in other groups fed partially by enriched feed showed tendency to the decrease of positive microbiological findings, and in particular, lower mortality was observed.

Some papers declare, that inclusion of a live yeast culture in weanling piglet diets affected intake and performance, however did not alter tested intestinal microflora or concentrations of fermentation products (3); in our trial average daily gain was higher in the control group, the greatest mortality in this group enables comfortable udder feeding and growth to the lower number of surviving piglets.

Presented results declare impact of addition of *Saccharomyces cerevisiae* in the feed on reduction of the number of ETEC strains in feces and lower pre-weaning mortality in the treated groups of sows and piglets.

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VACCINATION OF PIGLETS AGAINST *Escherichia coli*: EFFECTS ON NURSERY PERFORMANCES

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Introduction

Escherichia coli related diseases have increased over last years due to growth promoters antimicrobials ban and heavy metal restriction in feeds, as well as decrease in productivity[1,2]. In Spain, *E. coli* diarrheas pathocronia have moved from nursery to early finishing. The classical prevention and control have been based on sows vaccination and antimicrobial prophylaxis. Recently, a new vaccine, Colidex® (Farcovet, Spain) focused on piglets has been launched. The aim of this study was to assess the effect of piglet's vaccination against *E. coli* on health and performance during nursery.

Material & Methods

Six thousands piglets, from a 950 sows unit, were vaccinated and revaccinated at 10th and 20th days of age (V10-20; n=1,800), 10th and 30th days of age (V10-30; n= 1,800) and 20th and 30th days of age (V20-30, n= 1,800) were introduced in a finishing unit, having each one an unvaccinated control group (C10-20, n= 2,400; C10-30, n= 1,800; and C20-30, n=1,800). All groups were reared under same conditions and feeds, and in the same unit.

The production was made under three-sites-production system, being breeders, nursery and finishers in the same geographical area.

Mortality during nursery, causes of death (grouped in respiratory, enteric and wasted), enteric and respiratory diseases outbreaks, drug use and piglet total cost were recorded for each experimental group.

Results

The results regarding mortality and disease outbreaks appear in table 1:

Group	Mortality (%)	Enteric outbreaks	Respiratory outbreaks
V10-20	1.53	0	4
C10-20	3.64	2	5
V10-30	2.92	0	3
C10-30	3	1	0
V20-30	2.82	0	1
C10-30	1.17	0	0

The main difference was between V10-20 and C10-20 with a mortality decrease of 58%. In fact, the V10-20 group showed the lowest mortality all over the experience.

The results regarding causes of death appears in table 2:

Group	Mortality by enteric causes (% over total)	Mortality by respiratory causes (% over total)	Mortality by wasting (% over total)
V10-20	0	10.34	24.14
C10-20	11.76	0	38.24
V10-30	0	8.57	42.86
C10-30	5.56	0	55.56
V20-30	0	0	75
C10-30	0	0	42.86

The main cause of death was wasting, showing the lowest percentage the V10-20 group.

The piglets total cost was 41.75 vs. 42.28 € for V10-20 and C10-20, respectively; being the only groups with differences in production cost (0.5 € per produced piglet).

Discussion & Conclusions

The direct vaccination of piglets is a new approach to control *E. coli* related diseases. In this work we have assessed as the best protocol vaccination at and revaccination 10th and 20th days of life.

The main differences between vaccinated and control group were lower mortality, absence of enteric outbreak and decrease of wasted animals over total mortality, resulting all these effects on a production cost reduction of 0.5 € per piglet. To analyze all data it should be taken into account that different batches were reared in different seasons, but each vaccinated group had a control group at same time.

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VACCINATION OF PIGLETS AGAINST *Escherichia coli*: EFFECTS ON FINISHING PERFORMANCES

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Introduction

Escherichia coli related diseases have increased over last years due to growth promoters antimicrobials ban and heavy metal restriction in feeds, as well as decrease in productivity[1,2]. In Spain, *E. coli* diarrheas pathocronia have moved from nursery to early finishing. The classical prevention and control have been based on sows vaccination and antimicrobial prophylaxis. Recently a new vaccine, Colidex® (Farcovet, Spain) focused on piglets has been launched. The aim of this study was to assess the effect of piglet's vaccination against *E. coli* on health and performance during finishing.

Material & Methods

Animals vaccinated and revaccinated at 10th and 20th days of age (V10-20; n=1,200), 10th and 30th days of age (V10-30; n= 1,200) and 20th and 30th days of age (V20-30, n= 600) were introduced in a finishing unit, having each one an unvaccinated control group (C10-20, n= 2,400; C10-30, n= 1,200; and C20-30, n=1,200). All groups were reared under same conditions and feeds, and in the same unit. The production was made under three-sites-production system.

During finishing the parameters recorded were: mortality (M), feed conversion ratio (FCR), average daily gain (ADG), average initial weight (AIW), average slaughter weight (ASW), average days in feed (ADF), unmarketable animals (UA) and kilogram cost (KC).

Results

The results for productive parameters appear in table 1:

Table 1: Results recorded for M, FCR, ADG, AIW and ASW.

Group	M (%)	FCR (Kg)	ADG (g)	AIW (Kg)	ASW (Kg)
V10-20	6.67	2.75	740	21.03	108.2
C10-20	8.2	2.89	726	20.16	107.6
V10-30	8.51	2.75	660	22.10	102.6
C10-30	10.33	2.78	630	23.22	107.8
V20-30	7.92	2.91	630	20.70	106.6
C10-30	7.08	2.81	650	24.15	113.2

The results obtained for ADF, UA and KC are shown in table 2:

Table 2: Results recorded for ADF, UA and KC

Group	ADF	UA (%)	KC (€)
V10-20	118	5.36	1.044
C10-20	121	6.28	1.066
V10-30	118	4.39	1.068
C10-30	135	1.77	10.36
V20-30	135	1.9	1.08
C10-30	137	4.17	--

The main difference found was in M (-18.65%), FCR (-4.84%) and UA (-14.6%), resulting all in a reduction of KC (-2.06%), and meaning a save of around 1.76 € per finished pig, compared with C10-20.

Discussion & Conclusions

To analyze all data and obtain conclusions it should be taken into account that different batches were reared in different seasons, but each vaccinated group had a control group at same time.

We have found differences for all parameters comparing V10-20 and C10-20. The FCR was equal for V10-20 and V10-30 but the first batch was reared in winter and the second in spring. The seasonal variation of FCR is South-East Spain is very deep, so the improvement of FCR in V10-20 is relatively higher, compared with batches reared in spring or summer. We have calculated the standard deviation for FCR in this population to be 93 g, so the difference of 137 g between groups is significant.

The differences found are not constant in the other batches. The reduction of 2% in production cost during finishing is the most interesting value found.

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VACCINATION OF PIGLETS AGAINST *Escherichia coli*: LESIONAL STAGE OF GUT AND PRESENCE OF *Lawsonia intracellularis, Brachispyra hyodisenteriae* AND *Samonella spp.* AT SLAUGHTER

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Introduction

Escherichia coli is worldwide present in all farms. This pathogen is known to interact with other enteric pathogens, resulting in the Porcine Enteric Complex. Recently, a new vaccine, Colidex® (Farcovet, Spain) focused on piglets has been launched. The aim of this study was to assess the lesional stage of gut and mesenteric lymph nodes at slaughter, and the determination of infection by *Lawsonia intracellularis, Brachispyra hyodisenteriae* and *Salmonella* spp. in fresh samples.

Material & Methods

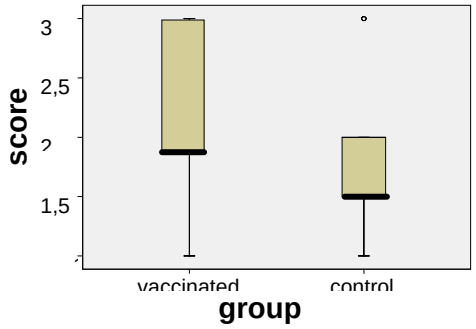
Samples of 20 pigs vaccinated and revaccinated at 10th and 20th days of age (V10-20) against *E. coli*, and 20 control unvaccinated animals (C10-20) were collected at slaughter. A piece of tissue was fixed in buffered formalin and an adjacent piece was frozen to develop histopathological and molecular diagnosis, respectively. Fixed tissues were wax embedded and microscopically evaluated for cellular infiltrate (categorical score, 0=no infiltrate, 1= slight, 2= moderate, 3=severe infiltrate, respectively). Acid nucleic from frozen pieces were isolated by conventional technic and a PCR for *B. hyodisenteriae* and *Salmonella* spp. was performed as previously described[1]. *L. intracellularis* was detected by means of a q-PCR[2].

Results

The lesions observed were lymphoid depletion in Peyer's patches (3 vaccinated and 3 unvaccinated animals), and slight edema, eosinophiles lyphocites and plasmatic cells infiltrating small gut mucosa in most of the animals. The median for infiltrate score is shown in figure 1. For infiltrate score, there was a significant differences by means of a Mann-Whitney's U test (p=0.028), but the highest median lesional score was found in vaccinated group.

As regards molecular determinations, on one hand none sample was found positive for *B. hyodisenteriae* or *Salmonella* spp. at slaughter. On the other hand, *L. intracellularis*, was found in 40% and 85% (p<0.001) of ileum samples from vaccinated and control animals, respectively, and in 25% and 65% (p=0.004) of colon samples from each group, respectively.

Figure 1: Boxes diagram for lesional score. The black thin line correspond to the median



The quantification of *L. intracellularis* showed a mean number of copies by gram of 64 and 27.4 x 10^6 for vaccinated and control group, respectively.

Discussion & Conclusions

The difference in infiltrate score still is under research but could be the result of continuous stimulation with *E. coli* in vaccinated animals. There was a very important difference in prevalence and density of infection by *L. intracellularis*, and it suggest that protection against *E. coli* could enhance the immune protection against *L. intracellluaris*, resulting in a better enteric health. These findings are related with an improvement of performance in finishing (especially in feed conversion ratio) detected for vaccinated group.

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CHECKLIST APPROACH OF THE HERD-SPECIFIC *SALMONELLA* CONTROL PROGRAM ON BELGIAN ASSIGNED RISK FARMS

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Introduction

Salmonella is considered as one of the most important food borne pathogens that has potential implications for human health (1). In a regulation to control *Salmonella*, the European Commission has set deadlines for its Member States to start *Salmonella* surveillance programs in the different livestock species that contribute to the risk of food borne infections in humans. Since 2005, the Belgian Federal Agency for the Safety of the Food Chain (FASFC) installed a National *Salmonella* surveillance and control program in pigs (SAP) which became compulsory by means of a Royal act in July 2007 (2). In a first stage of the SAP, the FASFC aimed to identify a maximum of 10% of the pig farms with high levels of *Salmonella*-specific antibodies. Since July 2007, risk farms are identified as farms with a mean S/P-ratio equal or higher than 0.6 for 3 successive sampling events. Once pig farms are identified as risk farms, they need to develop a herd-specific *Salmonella* action plan in order to improve their status. Therefore, a *Salmonella*-specific checklist was developed in order to point out some important and critical 'hazard' points on these farms. The outcome of these checklist is useful for identifying risk factors on these risk herds as well as a general sensitizing tool for the whole pig sector.

Materials and methods

Between July 2007 and January 2010, 776 Belgian pig farms have been assigned as *Salmonella* risk farms. These farms need to fill in a checklist, which is a written questionnaire including obligatory topics related to (i) general hygiene and management, (ii) biosecurity, (iii) animal management, (iv) water, (v) feed, (vi) veterinarian and zootechnical guidance and (vii) management of transport of fattening pigs to the slaughterhouse. Each topic contained approximately 10 questions pointing out the most important measures that can be taken to control *Salmonella* infection on farm level. The questions were designed by Belgian *Salmonella* experts and the questionnaire was pre-tested by different external veterinarians.

Results

Results obtained from 688 questionnaires from assigned *Salmonella* risk farms are summarized (Table 1).

Table 1. Results of 688 questionnaires from Salmonella risk farms

Topic	Question	YES
I	Using seperated material for sows and fattening pigs?	65
	Disinfection of boots during entering and leaving barn?	48
	Cleaning and disinfection when compartment is empty?	61
II	Registration of visitors?	12
	Are drivers and helpers prohibited to enter the barn?	88
	Are rodents consistently challenged?	87
	Pets are forbidden in the barns?	64
	Birds can't enter the barns?	68
III	Salmonella-status of piglet supplier is known?	54
	Are gilts raised separate from the fattening pigs?	48
	Do you use a group management system?	43
	All in-all out in nursery?	84
	All in-all out in battery?	64
	All in-all out in pre-fattening stage?	56
	All in-all out in fattening stage?	61
IV	Water supply: minimum annual cleaning and disinfection?	34
	Acidification of the water for sows?	9
	Acidification of the water for weaners?	17
	Acidification of the water for fattening pigs?	13
V	Feed: pellets or meal versus wet feed for fattening pigs?	82 *vs.* 18
VI	Clinical Salmonellosis in the past 2 years?	9
	Deworming in fattening pigs?	91
VII	12 to 18 hours fastening before charging?	96
	Transporters are not allowed to enter barn?	84
	Cleaning and disinfection of charging area?	73

Discussion

Proper cleaning and disinfection of the barns needs to be a standard work on pig farms. Compared to previous results of the checklist analysis, little improvement has been observed (3). Control of people, pets, and rodents entering the stables needs to be raised. Due to the implementation of group management systems and all-in all-out, higher levels of hygiene and biosecurity will be acquired on the risk farms.

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RELATIONSHIP BETWEEN SEROLOGICAL STATUS OF SOWS AND THE ASSIGNMENT AS *SALMONELLA* RISK FARM IN BELGIUM

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Introduction

Salmonella is considered as one of the most important food borne pathogens that has potential implications for human health (1). To control *Salmonella* at the pre-harvest stage, the implementation of a surveillance and control program has been established in the different EU Member States. Since 2005, the Belgian Federal Agency for the Safety of the Food Chain (FASFC) implemented a National *Salmonella* surveillance and control program in pigs, the *Salmonella* Action Plan (SAP), which became compulsory by means of a Royal act in July 2007 (2). Since July 2007, Belgian pig farms can be assigned as *Salmonella*-risk farms, based on serological analysis of blood samples collected from the fattening pigs.
This study was conducted to evaluate the serological status of the sows on *Salmonella* risk farms compared to non-assigned farms.

Materials and methods

With a 4-month interval, every Belgian pig farm needs to collect blood samples from 12 fattening pigs for the National Aujeszky-disease monitoring program. All samples are analyzed using an indirect LPS-*Salmonella* ELISA (Idexx). Since July 2007, farms are identified as risk farms if the mean S/P-ratio, from 12 fattening pigs, is equal to or higher than 0.6 for 3 successive sampling events.
For this study blood samples (n = 1138) of sows were randomly collected on 100 different farrow-to-finish herds. To this end, 583 samples were obtained from 50 *Salmonella* risk farms, identified as farms with a mean S/P-ratio equal to or higher than 0.6 for 3 successive sampling events; and 555 samples were obtained from non-risk farms, identified as farms with a mean S/P-value lower than 0.2 for 3 successive sampling events.
A statistical analysis (Mann–Whitney–Wilcoxon test) was performed to compare the mean S/P ratio in both sow groups.

Results

The results show that the mean S/P ratio obtained from the sows of the *Salmonella* risk-farms (1.138 ± 0.026 SEM) was significantly higher ($P < 0.0001$) compared with the non-risk farms (0.702 ± 0.021 SEM). The mean S/P-ratio for the 1138 sows was 0.925 ± 0.018 SEM. In this study there was a presence of *Salmonella* antibodies in 98.7% of the sows. At a cut-off of S/P = 0,6 we found 63,6% of the sows to be positive.

Table 1. Number of samples sows and their mean S/P-value ± SEM for Salmonella in blood

	N sows	Mean S/P-value
Risk-farms	583	1,138 ± 0.026
Non-risk-Farms	555	0,702 ± 0.021
Total	1138	0,925 ± 0.018

Discussion and conclusions

Sows play an important role in the maintenance of Salmonella infections in farrow-to-finish herds (3). The increasing number of sows on a farrow-to-finish farm was recently identified as a risk factor associated with higher average S/P-values on a farm (4). In this study we could clearly show that the infection status of the sows plays a significant role in the assignment as a *Salmonella* risk farm in the Belgian *Salmonella* control program, which is based on sampling of fattening pigs. Control of Salmonella on farrow-to-finish herds is now almost only done by implementing measures in the fattening unit. It is clear that further studies are needed to evaluate intervention measures in the sow unit. Vaccination could be one of these intervention options.
In conclusion, it is clear that the role of the sows and their serological *Salmonella* status is a potential influencing factor for the assignment of *Salmonella* risk farms, which is principally based on sampling of fattening pigs.

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BACTERIOLOGICAL PREVALENCE IN FINISHING PIGS FARMS ASSIGNED AS *SALMONELLA* RISK FARMS BY SEROLOGICAL SCREENING

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Introduction

Salmonella is considered as one of the most important food borne pathogens that has potential implications for human health (Mao et al.,2003). The EU Zoonoses Regulation Nr 2160/2003 requires Member States to take effective measures to detect and control *Salmonella's* of public health significance. Since 2005, the Belgian Federal Agency for the Safety of the Food Chain (FASFC) installed a National *Salmonella* surveillance and control program in pigs, the *Salmonella* Action Plan (SAP), which became compulsory by means of a Royal Act in July 2007 (Anonymous, 2007). As in most European countries, the monitoring and surveillance program for swine Salmonellosis is based on serological techniques. Once pig farms are identified as risk farms, based on 3 consecutive serological results with S/P > 0.6, they need to install a herd-specific *Salmonella* action plan in order to improve their status. The knowledge of prevailing serovars of *Salmonella* by bacteriological methods is essential to develop and/or evaluate the serological method. The purpose of this study was to assess the serovars most frequently isolated on these assigned risk farms in Belgium and to evaluate the correlation between serological status and bacteriological prevalence.

Materials and methods

After assignment as *Salmonella* risk farm, the bacteriological herd status is examined using environmental samples through overshoes. On each assigned farm 4 pairs of overshoes were collected from the different weight categories: ≤ 40kg, 40-59 kg, 60-79 kg, ≥ 80 kg. Bacteriological analysis for *Salmonella* was performed by means of a standard enrichment method according to ISO 6579-Annex-D (MSRV). *Salmonella* strains were serotyped at the National Reference Laboratory for *Salmonella* (VAR), according to the Kauffman-White scheme.

Results

From January 2009 until January 2010, 888 samples (= pair of overshoes) were collected from 222 different pig farms. *Salmonella* was recovered in 262 (30%) samples. Thirteen different serovars were identified.

Table 1. *Salmonella* enterica serovars identified from *Salmonella* risk farms in Belgium in 2009

Serovar	N	% of all positive samples
Typhimurium O5+	103	39,3
Typhimurium O5-	76	29,0
O4 : I : -	20	7,6
Derby	28	10,6
Livingstone	3	1,1
Brandenburg	6	2,3
Panama	2	0,7
Infantis	1	0,3
Rissen	4	1,5
O4,5:I:-	5	1,9
Agona	1	0,3
O4:-:-	2	0,7
O4,5 : I :-	3	1,1
Typing in progress	2	0,7
Non-typable	6	2,3
TOTAL	**262**	

Discussion

Based on serological screening, in 57% of the assigned pig farms there is a confirmed excretion by bacteriological isolation. Based on these results, we could conclude that the observed correlation between bacteriology and serology is sufficient in the first stage of the SAP. However, continuous monitoring of bacteriological and serological results will be necessary in order to fine-tune and develop a credible SAP based on diagnostic tools.

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SEROLOGICAL SCREENING OF SALMONELLA IN PIG HERDS USING SERUM AND MUSCLE TISSUE FLUID (MEAT JUICE)

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Introduction

Salmonella spp. is still among the most important food-associated infectious agent of intestinal diseases in humans. It is well known that many animals, especially pigs and poultry, may be infected but show no clinical illness. Such animals may be important to the spread of infection between herds and as sources of food contamination. EU legislations recommend national control programmes and such monitoring programmes have been set up in various European countries. Most of these programmes (in Germany and Denmark) use a meat juice antibody enzyme-linked immunosorbent assay test to identify positive pig fattening farms.

In this study, the serological ELISA was applied serum and meat juice samples.

Material & Methods

The investigations were carried out in a slaughterhouse in the west part of Romania. Animals from 12 farms were included in the investigations. Blood samples were taken into universal bottles when the pigs were bled out. A sample of diaphragm muscle was taken from each pig. At the laboratory the samples were frozen, then thawed. Meat juice was taken into uniquely identified tubes and stored at -20ºC until batch tested.

Serum and meat juice samples were tested by the HerdChek* Swine *Salmonella* Antibody Test Kit (IDEXX Switzerland AG).

Results

Table 1 summarizes the number of all farms tested (F1 to F12), number of pig tested in the slaughterhouse per farm, number and percentage (%) of positive serum samples and number and percentage (%) of positive meat juice samples.

The results relating to serology carried out on serum shows that from 120 serum samples tested 60 (50%) were positive.

A total of 37 of the 117 (31.62%) meat juice samples gave positive results. Only one farm (F6) gave negative results in serum samples and in meat juice samples too.

In six from 12 farms we obtained positive results > 60% (F1, F4, F9 -90%, F10 – 80%, F5 – 70% and F12 – 60%). The results relating meat juice samples presented in table shows that the percentage of positive results are lower than serum samples, only in F1 the positive samples were high. Three farms (F7, F11, F12) had 50% positive results, four farms (F2, F3, F4, F9) had less than 30% positive results and three were negative.

Table 1
Salmonella ELISA for pig serum and meat juice samples from 12 farms

Name of farm	No of tested pigs	serum samples positive (total)	meat juice samples positive (total)
F1	10	9 (10)	8 (10)
F2	10	2 (10)	3 (10)
F3	10	4 (10)	3 (10)
F4	10	9 (10)	2 (10)
F5	10	7 (10)	0 (10)
F6	10	0 (10)	0 (10)
F7	10	3 (10)	5 (10)
F8	10	2 (10)	0 (10)
F9	10	9 (10)	2 (10)
F10	10	8 (10)	4 (10)
F11	10	10 (10)	5 (10)
F12	10	6 (10)	5 (7)
TOTAL	120	60 (120)	37 (117)

Discussion & Conclusions

A general shortcoming of antibody-based tests is that negatively interpreted antibody ELISA results do not always give a full guarantee that that animals are free from *Salmonella*. Contamination could have occurred shortly before the day of slaughter, during transport before an immune response can be measured.

Comparison between the ELISA results for serum and meat juice showed similar results, but differences amongst farms were relatively high.

The results of this work are in agreement with other studies, which suggested that further work must carry out on samples from a larger number of farms over a longer period.

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DETECTION OF SALMONELLA ANTIBODIES IN SERUM VERSUS MEAT JUICE FROM DIFERENT CARCASS LOCATIONS

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Introduction

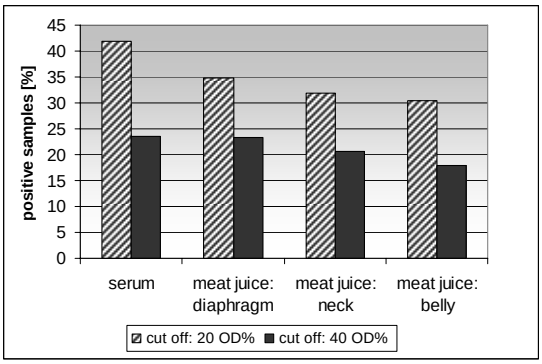
Following the Danish experience with categorizing finisher pig herds according to their risk level of being infected with Salmonella spp. using a serological monitoring (2), several EU member states have implemented national Salmonella control programmes based on random sampling of meat juice or serum for the detection of Salmonella antibodies. The German programme categorizes the herds after 60 samples per herd and year have been taken and tested for Salmonella antibodies. At present, the cut-off value for the decision "positive" or "negative" has been set at 40 OD%. The categories to which the herds are assigned to are: Category I (low risk): less than 20 % of the 60 samples are positive, Category II (medium risk): between 20% and 40% of the 60 samples are positive, and Category III (high risk): more than 40% of the 60 samples are positive. Until recently, the following protocol for taking samples at the slaughter line was: freezing and thawing a piece of meat from the diaphragm pillar and testing the resulting meat juice for Salmonella antibodies with a licensed mixed ELISA.. Over time, however, logistic considerations have led to slight changes of the original protocol: e.g. blood serum collected during the bleeding procedure instead of meat juice, or collecting meat samples from neck or belly muscles instead of the diaphragm pillar. These changes made investigations into the comparability of the different matrices (serum vs. meat juice) and of the different origin of the meat for producing the meat juice (diaphragm, neck and belly).

Material & Methods

To compare ELISA results of blood serum samples to meat juice samples from the three different origins 411 pig carcasses from more than 20 different pig herds were sampled at the slaughter line. Since for this study it was absolutely necessary to take the four samples per carcass from the same animal, every carcass got a special tattoo at the bleeding site. All samples were tested for Salmonella antibodies using the IDEXX HerdCheck®. The results of the four tests from the samples of 411 carcasses were analysed using a one-factorial variance analysis to quantify the variances between the matrices and the muscle origins, the significance was tested.

Results

The graph shows the proportion of positive samples using the cut-off 20 OD% and 40 OD%. In contrast to meat juice from the neck and the belly, the "diaphragm meat juice" results do not significantly differ from those of the serum samples.



Discussion & Conclusions

For the German Salmonella monitoring (currently using the cut-off value 40 OD%), both blood serum and meat juice can be used for the serological categorization of pig herds. However, there is a need to define the diaphragm muscle as the only carcass location as mandatory to assure that serum and meat juice results can be used likewise in the national data base for the monitoring programme.

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SALMONELLOSIS IN FATTENING PERIOD: RELATIVE IMPORTANCE OF SOWS IN TWO POINTS MULTIPLE-SITE PRODUCTION

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INTRODUCTION:

The importance of slaughterhouses and fattening farms are well established in swine salmonelosis as risk points in chain food production However, the role played by sows is not very clear. The aim of this study was to investigate the importance of sows in the prevalence of *Salmonella* infections during the fattening period in multiple-site production units.

MATERIALS AND METHODS:

Three two points multiple-site production farms were serologically evaluated. In each one, a breeding farm and some finishing facilities were evaluated. Forty serum samples were collected per farm; in breeding farms (Farms A to C) serum samples were taken from pregnant, suckling and weaned sows while in fattening farms samples were taken at the end of the fattening period. Serum samples were submitted to the laboratory, processed and tested by ELISA using a commercial test (Idexx herdchek Swine Salmonella®) according to manufacturers' instructions. Cut off selected for positive samples was 20%.

RESULTS AND DISCUSSION:

Salmonella seroprevalence was high among breeding farms. Percentage of positive samples was 97.5% in Farm A, 77.5% in farm B and 80% in farm C.

Regarding Farm A and B four fattening units (A1-A4 and B1-B4) were investigated while 3 units (C1-C3) receiving pigs from Farm C were selected. As show in the graphics, seroprevalence results from fattening units were very variable ranging from 0 to 97.5%.

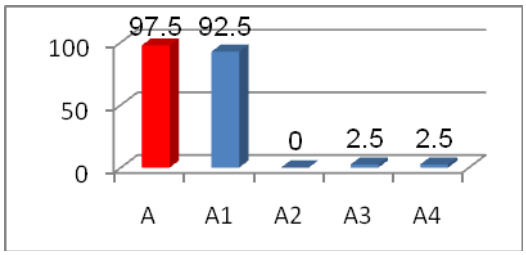

Fig 1. Breeding farm A (red bar) supplies pigs to fattening units A1 to A4 (blue bars).

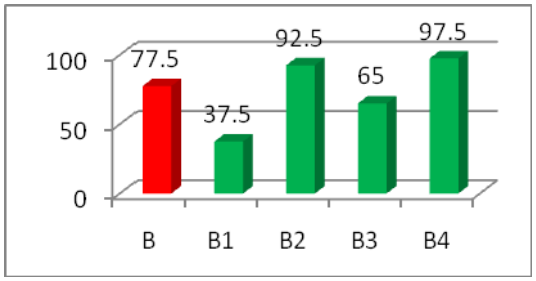

Fig 2. Breeding farm B (red bar) supplies pigs to fattening units B1 to B4 (green bars).

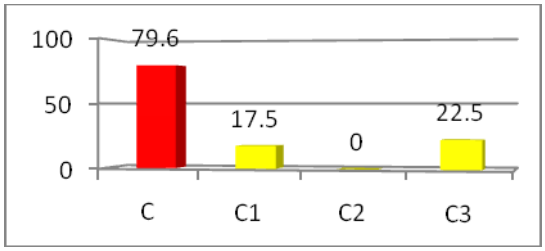

Fig 3. Breeding farm C (red bar) supplies pigs to fattening units C1 to C3 (yellow bars).

Our results reveal that in non-farrow to finish farms there is not a clear relationship between *Salmonella* infection in sows and finishers as previously proposed [1]. However, other studies have proposed an important role for sows as source of *Salmonella* infection for finishers [2]. Deeper studies are required in order to answer this question including molecular typing methods to compare *Salmonella* isolates.

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EFFICACY OF VACCINATION WITH AN INACTIVATED VACCINE TO REDUCE SALMONELLA PREVALENCE IN A PIG FATTENING UNIT

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Introduction:

Immune response stimulation by vaccines has been a useful mechanism stand up to pathogens [1]. In *Salmonella* live vaccines are the first choice, but they have several disadvantages like biosecurity, oral administration and higher prices, so we have decided to develop a vaccination trial in a swine farm using an inactivated vaccine of *Salmonella* Typhimurium, which is easy to produce and cheaper than lives vaccines.

Materials and methods:

The vaccine was prepared from a culture of *Salmonella* Typhimurium DT104 (10^9 UFC/ml) inactivated by formol and using hydroxide aluminum $Al(OH)_3$ as adjuvant. A pig fattening unit (3000 animals) infected by *S.* Typhimurium and *S. 4,5,12:i:-* was chosen for the experiment. Within the farm, animals were housed in two different barns. Pigs from one of the barns, vaccinated group, were injected intramuscularly 2 ml of the inactivated vaccine at the beginning of the fattening period (70 days of life approximately) and 25 days later. Pigs from the second barn served as control group and receive no treatment. Both groups were monitored during the fattening period by collecting serum and fecal samples that were used for serological and bacteriological detection of *Salmonella* infection. Moreover, cecal content, mesenteric lymph nodes and blood samples were collected after slaughter from both groups. In each sampling forty animals per group were included. Bacteriological analyses were made using EN-ISO 6579:2002/Amd 1:2007: Microbiology of food and animal feeding stuffs- Horizontal method for the detection of Salmonella spp. in animal feces and in environmental samples from the primary production stage. Serological samples were tested with Idexx herdchek Swine Salmonella kit® according to manufacturers' instructions and using 40 % PI as cut-off.

Results:

The percentage of *Salmonella* shedders was significantly lower (χ^2=67,5 p<0.001) among vaccinated animals as compared with control pigs (18 % - 65 % in control group versus 0 % - 7.5 % in vaccinated group). Abattoir study also revealed significant differences in the prevalence of *Salmonella* positive samples among cecal content (χ^2=33,43, p<0.001) (55 % versus 15 %) and mesenteric lymph nodes(χ^2=20,20, p<0,001) (55 % to 22.5 %). Moreover, significant differences (χ^2=49,66 p<0.001) in the percentage of seropositive animals were also detected al the end of the study (88 % of seropositive animals in control group versus 44 % in vaccinated group.

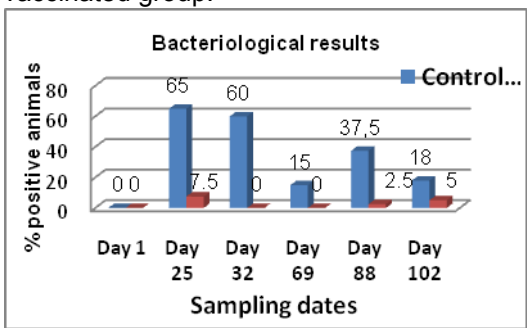

Figure 1. Percentage of fecal shedders in experimental (vaccinated on day 1) and control groups

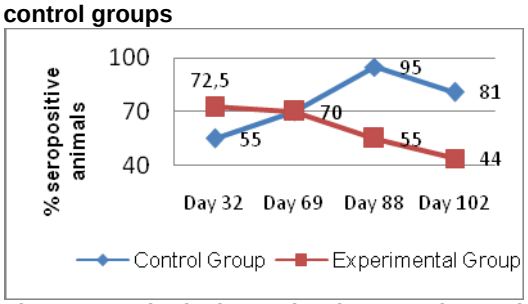

Fig 2. Serological results in experimental (vaccinated on day 1) and control groups.

Discusion:

Despite of live vaccines and its cell mediated response provide a better protection [2,3], our study demonstrates that inactivated vaccines can be useful protecting swine salmonellosis. Further investigations are needed to corroborate these results in other farms even which other serotypes less close to Typhimurium. Its disadvantage is that its seropositivity makes difficult to use it in control programs.

References:

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EVALUATION OF A COMMERCIAL ACID MIXTURE ADMINISTERED IN DRINKING WATER AS A CONTROL MEASURE IN SWINE SALMONELOSIS

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Introduction:

Salmonella control programs have been developed in swine farms from several European countries [1]. Those farms with a high level of contamination will have to establish control measures to reduce the number of *Salmonella* infected animals [2]. We have carried out an interventional study in a pig fattening unit to assess the effectiveness of an acid treatment administered in drinking water for the control of salmonellosis.

Materials and methods:

The study was performed in a pig fattening unit infected by *Salmonella* Typhimurium housing 2960 pigs distributed in two barns (1480 pigs each). One of them was chosen as control group and was not vaccinated while the other was chosen as experimental group (vaccinated animals). Animals from experimental group were administered a commercial acid, Acidvall®, composed of lactic acid (56 %), formic acid (23 %), propionic acid (13 %) and acetic acid (5 %), that was added to drinking water during the last 40 days of the fattening period at a concentration of 0.035%. Within each group, 40 animals were randomly selected and sampled through the experiment. Fecal samples and serum were collected the first days of the fattening period, at the beginning of the treatment, 20 days after as well as at the last day of treatment (previous day to slaughter). Moreover, cecal content and ileocecal lymph nodes were collected from pigs of both experimental groups at the slaughter. Samples were submitted to the infectious animal diseases laboratory and processed. Bacteriological analyses were made using EN-ISO 6579:2002/Amd 1:2007. Serological samples were tested with Idexx herdchek Swine Salmonella kit® according to manufacturer's instructions.

Results:

Results showed significant differences in bacteriology (χ^2=21,4 p<0,001) and serology (χ^2=71,45, p<0,001) between groups at the end of the fattening period (17.5 % shedders and 27.5 % seropositive pigs in treatment group compared to 50 % shedders and 87.5 % seropositive pigs in control group). Similar results, near to significance, were found out at the abattoir where *Salmonella* was detected in cecal content in 47.5% of the treated pigs versus 65 % of the control animals. Differences were also found in the prevalence of positive animals in ileocecal lymph nodes 55% of positive in treatment group to 70% in control group.

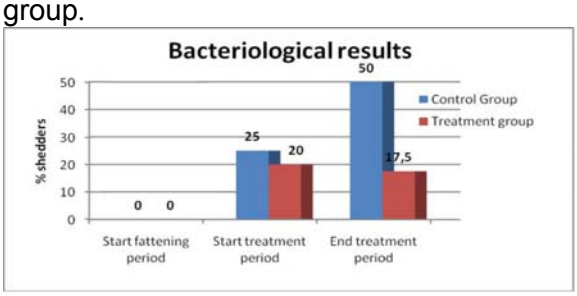

Figure 1. Bacteriological results, fecal samples, during fattening period.

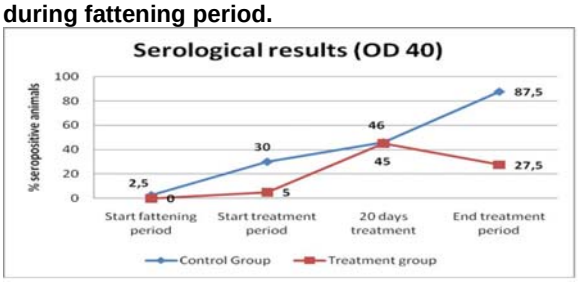

Figure 2. Serological results during fattening period. Cut off 40%.

Discussion:

Several studies have demonstrated that organic acids can be useful to reduce Salmonella contamination in swine farms [2,3,4]In the present study the treatment, which was designed taking into account treatment economic feasibility (0.035 %, during forty days) has been able to demonstrate a reduction of the number of *Salmonella* shedders. Moreover, the number of *Salmonella* seropositive animals was also reduced. Despite that this results are promising further studies are required to confirm them.

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EFFECT OF PRE-SLAUGHTER DIET SUPPLEMENTED WITH A PROTECTED SOURCE OF FORMIATE AND CITRIC ACID ON THE PREVALENCE OF *SALMONELLA* IN CARRIER PIGS

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Introduction. *Salmonella* spp. infection represents a relevant problem for pig industry. Pigs are often asymptomatic carriers of *Salmonella* throughout different breeding stages until slaughter, representing a potential source of infection and a risk for human health. Following Regulation (EC) 2160/2003, the European Food Safety Agency (EFSA) published in 2008 the first results of a baseline survey on the prevalence of *Salmonella* in slaughter pigs in the European Union between 2006-2007 [1]. The average prevalence of *Salmonella* in swine mesenteric lymph nodes in the EU was 10,3%. In Italy the prevalence was reported to be higher and equal to 16,5%. In the second part of the report [2], after the evaluation of risk factors for *Salmonella* infection in slaughter pigs, control strategies at herd level emerge as a requirement to control *Salmonella* contamination in pigs and meat products. Depending on the epidemiological situation, each Member State should establish a National Control Plan to achieve this objective. The knowledge of effective tools to reduce *Salmonella* prevalence at herd level should be acquired. To support this objective a field trial was carried out to determine the effectiveness of feed supplement based on a protected source of formiate and citric acid (Formyl™) as a complementary tool in reducing *Salmonella* carrying-pigs at slaughtering.

Material & Methods. The study was performed in a farrow-to-finish herd located in Emilia-Romagna (Northern Italy) with high rate of seropositivity for *Salmonella.* The treated group was fed with the finishing diet with 4 Kg of Formyl™ per tonne of feed for at least the last 30 days before slaughter, while the control group was given a commercial finishing diet. Three batches of treated and control pigs were included in the study, and were weekly sent to the slaughterhouse. The treatment started 30 days before the scheduled date for slaughtering of the first two batches. Consequently, animals of the treated group received the experimental diet for a minimum of 30 days and a maximum of about 50 days. The farm did not perform massive-antibiotic treatments during the study. In case of parenteral therapy, treated animals were marked and excluded from the study. Transport to the slaughterhouse took about 2 hours and lairage interval lasted between 1-4 hours. The caecal content (CC) and mesenteric lymph-nodes (LN) were sampled from 104 treated and 100 control pigs after slaughtering. Samples were sent to the laboratory in chilled containers within 2 hours after slaughtering and submitted to *Salmonella* analysis using the standard ISO 6759:2002/Amd 1:2007 method. Pigs were considered as carriers on the basis of the presence of *Salmonella* in either CC or LN. The differences between the observed *Salmonella* incidence in control and treated pigs were evaluated by X^2 test.

Results. In the control group bacteriological investigations for *Salmonella* on LN showed a prevalence of 43,00%, while in the treated group this incidence was reduced to 28,85%. The difference of 14,15% [IC95%: 1,11%-27,19%] in the proportion of positive samples between the control and treated groups was statistically significant ($p<0,05$). The *Salmonella* occurrence in the control group was 1,5 (43,00/28,85) times higher than in the treated group. In CC of the control group 41,00% of samples were *Salmonella* positive whereas in the treated group *Salmonella* was detected in 24,04% samples. The difference of 16,96% [IC95%: 4,29%-29,63%] between the groups was also statistically significant ($p<0,01$). The relative contamination of the CC in the control group was 1,7 (41,00/24,04) times higher.

Discussion & Conclusion. In this trial statistically significant differences were observed in the prevalence of *Salmonella* in CC and LN samples from control and Formyl treated group, with a treatment period of at least 30 days before slaughtering. The effect of the product on the reduction of *Salmonella* incidence was stronger in CC than in LN. This because Formyl™ is targeted to be active at the intestine level. The results obtained from this field trial put into evidence that the add of protected organic acids to the diet, for a period of at least 30 days before slaughtering, can be considered a valid tool for the reduction of *Salmonella* infection in pigs.

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DETECTION RATES OF *SALMONELLA* IN LAIRAGE, INTESTINES AND ON PIG CARCASSES IN FIVE SLAUGHTERHOUSES

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Introduction

In 2008, 3944 *Salmonella* infections in humans have been registered in Belgium, of which *Salmonella* Typhimurium (57%) was the most common serotype (1). The aims of this study were to investigate the prevalence of *Salmonella* contamination in the lairage area and along different steps of the slaughter line and to identify possible critical control points.

Material and Methods

Five Belgian slaughterhouses (slaughter rate of 170-580 pigs/hour) were visited twice. Faecal samples from pigs of different batches were taken at the lairage area using overshoes. At exsanguination, 30 pigs were individually identified (total: 30 pigs × 5 slaughterhouses × 2 = 300 pigs) and swab samples of the oral cavity (Oral C) were taken. The same 30 pigs were further investigated along the slaughter line. Carcass swabs were taken after polishing (Sa.Pol), after splitting (Sa.Split) and after first chill (Sa.f.chill) as well as content of duodenum (Duod), ileum and rectum and mesenteric lymph nodes (LN) of the corresponding pigs. Water samples were also taken from the scalding tank prior and during slaughter. All the samples were submitted to *Salmonella* isolation using standard procedures. Associations between *Salmonella* contamination levels at different points of the slaughter line were calculated using Fishers' Exact Test in SPSS 17.0. *P*-values <0.05 were assumed significant.

Results

In total, 276 samples (14.4%) were *Salmonella* positive, with 48.2% of the pigs positive in at least one sample. The lairage area was highly contaminated (average=52.8%) with a large variation (0-100%) among the different slaughterhouses. Contents of ileum and mesenterial lymph nodes were positive in 23.1% and 17.7% of the samples, respectively. The samples of the scalding tank water were all *Salmonella* negative. Table 1 gives the most important findings of the odds ratio calculation.

Table 1: Odds ratios between the contamination level of the different samples taken at slaughter

	Lairage	Sa.Pol	Sa.Split	Sa.f.chill
Oral C.	42.6*	9.8*	20.6*	4.1
Sa.Pol	22.2*		28.5*	38.3*
Sa.Split	10.7*	28.5*		10.4*
Sa.f.chill	Np	38.3*	10.4*	
Duod.	4.7*	1.5	2.7	Np
Ileum	1.9	0.6	1.7	0.8
Rectum	7.2*	1.3	4.4*	Np
LN	4.3*	0.9	1.4	3.2

*: significant/ Np: not possible due to a 0-value

Discussion and conclusion

Salmonella was commonly present in the lairage area, in the pigs' intestines and on the carcasses along the slaughter line. The results clearly indicate that a *Salmonella* contaminated lairage area is more associated with positive carcasses than with positive intestines. There was one slaughterhouse where all samples of the overshoes from the lairage area tested negative. The lairage area here consisted of a slatted floor allowing a minimum of contact with the feces. A good fasting procedure, prevention of accidental cutting of intestines and improved cleaning and disinfection of the lairage area are important factors to prevent or minimize contamination of the lairage area. The risk of a positive carcass further increases when the carcass tested positive on a previous sampling point. There was no significant association between the contamination of carcass samples and that of the intestines, except for swabs taken after splitting and rectum content. This confirms the fact that cutting out the rectum is a critical point for *Salmonella* contamination at the slaughterline.

Reference

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FOLLOW-UP STUDY ON THE SEROLOGICAL PREVALENCE OF *YERSINIA ENTEROCOLITICA* IN FATTENING PIG HERDS

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Introduction

Yersinia (*Y.*) *enterocolitica* presents a common cause of human gastroenteritis (1). Pigs have been identified as the primary reservoir of pathogenic *Y. enterocolitica* and food products of porcine origin are important sources of human infection (2). Thus, reducing the prevalence in pig herds might reduce the risk for consumers. Prior to implementing a surveillance and control programme, it is important to gain knowledge of the dynamics and epidemiology of *Yersinia* infections in pig herds.

The objective of the study was to investigate the steadiness of serological *Yersinia* herd status over time.

Material & Methods

Based on their serological *Yersinia* herd status, a sub-sample of nine herds participating in a research project (ZiPP = Zoonoses in Pork Production) was selected for this study. Within-herd prevalence was determined by blood sampling of 30 slaughter pigs during exsanguination. A herd was assigned seronegative *Yersinia* status if no sample tested positive at a cut-off ≥ 20 OD %. Four negative herds and five herds with a within-herd seroprevalence ≥ 96.7 % were selected. The development of the *Yersinia* status was assessed by four subsequent sampling rounds over a period of a year. At each round, 30 samples from slaughter pigs were taken.

Blood samples were analysed for antibodies against virulent *Yersinia* strains by using *Yersinia* Outer Protein (YOP)-antigens (Pigtype® Yopscreen Pig ELISA, Labor Diagnostik Leipzig).

Results

Table 1 shows the within-herd prevalence of the selected herds during the different sampling rounds. Within the observation period, the initial negative herds 1, 2, and 3 showed a prevalence of 6.7 % maximum, whereas the prevalence of herd 4 shifted between negative status and a prevalence of 100 %.

Those herds which initially had a high serological prevalence did not change to a negative status during the observation period.

Table 1. Initial herd status and within-herd *Yersinia* prevalence of nine herds during the four sampling rounds (SR) within a year

		Prevalence during the follow-up study (%)			
	Initial herd status	SR 1	SR 2	SR 3	SR 4
Herd 1	neg.	neg.	3.3	6.7	neg.
Herd 2	neg.	6.7	neg.	3.3	neg.
Herd 3	neg.	6.7	neg.	neg.	neg.
Herd 4	neg.	76.6	3.3	100	3.3
Herd 5	100	96.7	96.7	100	100
Herd 6	96.7	96.7	93.3	96.7	96.7
Herd 7	96.7	80.0	90.0	56.7	96.7
Herd 8	100	93.3	46.7	100	100
Herd 9	96.7	83.3	96.7	40.0	96.7

Discussion & Conclusions

Raising pigs from breeding and multiplying herds free from pathogenic *Y. enterocolitica* might reduce the risk of fattening pigs being infected (3), because transmission of *Y. enterocolitica* is more likely from other infected pigs than from the environment (4). This might be the reason for the steady low prevalence of herd 1, 2, and 3. However, although herd 4 did not change the supplier during the investigation period, the prevalence varied between negative and high positive. Therefore, other sources of contamination must be taken into consideration.

References

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GASTRIC ULCERS IN 7–8-WEEK-OLD PIGS, FED BY VARIOUS FORMULAE OF PELLETED FEED

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Introduction

This experiment was focused on the gastric health in 7-8-week-old pigs fed by four different feed formulae. Pigs were from a herd of high health status, and their nursing period was well documented.

Material & Methods

Altogether 64 weaners, 29.5 days old on average, were divided in four feeding groups. In reference to the sex, breed and littermate, the pigs were mingled and allotted in four feeding groups and were housed in pens of two pigs. The four feeds in the experiment were: wheat-based with pellet diameter of 2.5 mm and 4.0 mm, and dehulled-oats-based with pellet diameter of 2.5 mm and 4.0 mm. The pigs were euthanized at the end of 25-day experiment. The feed consumption and the weight gain of the pigs were recorded, and the health status of the pigs was monitored daily. The stomachs were investigated at necropsy, the pH-value of anterior and posterior stomach, as well as dry matter content of stomach content was measured. The gastric lesions were scored by the scheme which differentiates apparently harmless lesions from those which undoubtedly cause pain and/or affect the normal eating (1).

Results

The main results from the judgement of gastric lesions are presented in Table 1. The smaller pellet size of feed correlated with the higher prevalence of severe gastric lesions in pigs ($p = 0.02$). Prevalence of gastric lesions was fairly similar in wheat- and dehulled-oats-based diets. The pH on pars oesophagea of the stomach was lower in the pigs fed diets with smaller pellets ($p=0.01$), as well as in pigs with severe gastric lesions. The dry matter content of the stomach content was lower in pigs fed by the smaller pellets ($p=0.001$). The feed conversion ratio among pigs with severe gastric lesions did not significantly differ from that of pigs with healthy or slightly altered stomachs. However, pigs with severe lesions had slightly lower growth rate during their nursing period than the pigs with no or slight lesions. The castrated males had more often severe lesions (50%) than the gilts (27%). The pigs from the first-litter sows had more often gastric lesions (53 %) than those from the litters of older sows (33 %).

Table 1. Gastric lesions in pigs in four feeding groups after 25-day experiment post weaning

Grain	wheat	wheat	oats	oats
Pellet	2.5mm	4.0mm	2.5mm	4.0mm
Severe lesions	8	3	9	5
No severe lesions	8	13	7	11
No of pigs	16	16	16	16

Discussion & Conclusions

The finely ground grain and/or small pellet size has been shown to be a risk factor for gastric ulcer in earlier studies (2,3). Low pH on pars oesophagea and low viscosity of stomach content by using finely ground grain have been identified earlier as well (2). Dehulled oats as main cereal instead of wheat did not prevent gastric ulcers. The hulls of oats in feed have been shown to protect against ulceration (4). The crude protein content in feed might have increased during decades due to breeding for faster gain of red meat; protein-induced HCl secretion might be one risk factor. The nursing period seems to include factors which expose suckling pigs to gastric ulceration. The correlation between stomach health of weaners and the amount of creep-feed intake before weaning might be worth of studying. If castration pain could cause enough stress to predispose the males to gastric ulceration is a question worth of further studies, as well.

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CASE REPORT OF A PERACUTE CARBON MONOXIDE POISONING IN NEWLY WEANED PIGS

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Introduction

Carbon monoxide intoxication in pigs, although not often observed, occurs in connection with dysfunction of heating devices and poorly ventilated compartments. Depending on the level of carbon monoxide in the air and the time of exposure it can take peracute to chronic courses. This case report describes the occurrence of acute carbon monoxide intoxication in weaned pigs. It points out the course of actions to approach the diagnosis.

Material & Methods

In February 2009, on a piglet production farm almost all piglets of one batch, which was stocked in one compartment, died within few hours after weaning. The pig farmer had not noticed any symptoms of disease at the point of weaning. In addition to a clinical observation of the piglets in all compartments of the stable, six of the dead piglets were subjected to a pathological and histopathological examination. Furthermore, a thorough examination of the environment of the animals was conducted, comprising a simulation of the climatic conditions in the unit on the day of re-stocking. Therefore the gas-fired heater, which was used to heat up the compartment on that day and had been maintained recently, was put into operation. After some hours, the concentration of carbon monoxide in the air of the compartment was measured via gas detection pump under strict safety precautions, supervised by experts from the local fire department.

Results

The examination of the animals in the barn did not give any clinical evidence; the piglets did not show any specific signs of disease. Feed and water could be excluded as source of the disease for the piglets had still not received feed, and the water was the same as in other compartments. During pathological examination of six piglets there could not be detected any abnormalities that could have explained the cause of death. Especially the colour of blood and tissues could be ascertained as physiological. Also the findings in the histopathological examination were unspecific. The measurement of the carbon monoxide content in the air during simulation of the climatic conditions in the compartment revealed a concentration of 3000 ppm.

Discussion & Conclusions

Contrary to expectations, pathological as well as histopathological examination did not contribute to the diagnosis of carbon monoxide intoxication. None of the symptoms described in literature as typically being related to carbon monoxide poisoning, such as cherry-red blood, rose-coloured tissues or meningoencephalitis, could be detected in the examined animals. The diagnosis was only made after intensive examination of the environment, including running the gas-fired heater which was used in the relevant compartment. The measured concentration of 3000 ppm was clearly above the minimum lethal concentration of 1000 ppm. This demonstrates that in cases of suspected carbon monoxide intoxication it is indispensable to take even more elaborate diagnostic measures like the described reconstruction of the climatic conditions in the compartment: In the absence of clinical and (histo)pathological findings this might possibly be the only successful way to confirm and prove the diagnosis.

The excess in carbon monoxide in the unit was attributed to a malfunction of the gas-fired heater. Especially in combination with very low ventilation rates as common during winter time, this can easily lead to high carbon monoxide levels in the air of the compartment. Therefore veterinarians should urge pig farmers to exercise due care in the use of these devices, not only for reasons of animal welfare but also for their own safety.

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PREVALENCE AND RISK FACTORS FOR LAMENESS AND CLAW LESIONS IN SOWS KEPT IN TWO TYPES OF GROUP HOUSING

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Introduction

Claw lesions and lameness in sows are an important welfare concern as well as a cause of considerable economic loss. These problems are more common in group housing than in individual housing systems[1]. Given that group housing for pregnant sows will become mandatory in the EU from 2013 onwards, the aim of the present study was: 1) to determine the prevalence of lameness and claw lesions in sows, and 2) to analyze whether type of group housing and sow related factors were associated with lameness and claw lesions.

Materials and methods

Eight Belgian pig herds with group housing of pregnant sows were selected. In four herds, sows were housed in pens with electronic sow feeders (static groups), in four other herds, free access stalls were used (table 1).

Table 1: General characteristics of the 8 sow herds (B1-B8)

	B1	B2	B3	B4	B5	B6	B7	B8
Herd size	200	750	230	200	140	160	700	280
N° of assessed sows	55	69	85	47	13	53	58	41
Breed	T	T	D	H	RS	J	D	J
Mean parity assessed sows	1.8	2.8	4.5	4.0	2.8	3.6	3.2	3.2
# week batch production system	3	2	4	3	1	5	4	3
Type of group housing	FA	FA	FA	FA	ESF	ESF	ESF	ESF
Culling % due to locom. disorders	6	0.6	3	4	9	15	12	7
Use of bedding	-	-	-	-	-	-	-	+

ESF: electronic sow feeder, FA: free access stalls T: Topigs, H: Hypor, D: Danbred, J: JSR, RS: Rattlerow-Seghers

At the end of gestation, sows were investigated for lameness by visual judgement of their gait (lame or not lame). Within 3 days after farrowing, possible lesions of the claws of the hind limbs were scored using a standardized scoring system[2]. Information about feed, housing conditions, especially floor properties, and culling (strategy) was collected as well as information about parity and breed of each sow. Logistic regression analysis was used to evaluate risk factors (type of group housing, breed, parity and lesion score) for lameness. The risk factors for claw lesions were analyzed by linear mixed model analyses. All analyses were performed using SPSS version 16.00. A *P* value <0.05 was considered significant.

Results

Of the total of 421 sows that were investigated, 9.7% was lame (min. 2.4%, max. 23.1%). No significant difference for lameness was found between the two types of group housing (mean prevalence: 7.4% in FA, 13% in ESF). The prevalence of lameness significantly increased with increasing parity of sows. There were significantly more lesions found on lateral claws than on medial claws, especially the most frequently adjacent to the wall and heel area. Only lesions on the heel area differed significantly between both types of group housing with higher scores in free access stalls (mean score: 8.3 versus 7.7). The prevalence of side wall lesions and overgrown (dew-)claws increased with increasing parity. Breed was not significantly associated with lameness or claw lesions in this study.

Conclusions

Lameness and claw lesions in sows housed in group during gestation are commonly found in Belgian pig herds and their prevalence was higher in older sows. This indicates that a balanced parity distribution of sows is not only important for productivity reasons but also from a health and welfare point of view. Further research will focus on ways to control or prevent claw lesions in group housed sows.

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CT/MR IMAGING FINDINGS AT JUVENILE PIG WITH LORDOSIS AND KYPHOSIS

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Introduction

Lordosis and kyphosis ("humpy-back" or shortly only kyphosis) in pigs is a visual condition in which the thoracic spine appears concave and the lumbar region shows convex deformity. Since the first description by Penny and Walters in 1986 [1], the number of cases has been growing worldwide. The juvenile kyphosis (type I) [1,2] is aetiologically distinct from other conditions of the disease which affects the growers and finishers (type II & III) [3,4]. All the kyphoses also raise the question of welfare and economy. The juvenile kyphosis is not apparent at birth and its initiation has been debated.

Material & Methods

A 35 days old piglet examined originated from a farm where the clinical condition of kyphosis appeared in a growing number. The severely affected case became manifest at 7 days of age. In vivo cross sectional digital images of the piglet's vertebrae were acquired using computed tomography (CT) and magnetic resonance imaging (MRI) scanners. MR cross sectional imaging was performed on a 1.5T MR scanner (Magnetom Avanto, Siemens). A phased array torso coil, comparable with the size of the piglet was used for imaging purposes. A sagittal plane, T2 weighted turbo spin echo (TSE) pulse sequence was obtained with a slice thickness of 4 mm.
The CT scans were performed using a multislice CT scanner (Somatom Emotion 6, Siemens) using the following settings:130 kV tube voltage, 160 mm field of view and 1.25 mm slice thickness. Sagittal 3D maximum intensity projection (MIP) reconstructions were produced using the transversal cross sectional images. Throughout the examinations the piglet was anesthetised using isofluran gas (vol% 2).
Following the in vivo investigation, the piglet was euthanised, fixed in buffered 4% formaline solution and gross pathological examination was carried out. Following removal of the visceral organs the spinal column was deep freezed. The deep freezed vertebraes were longitudinally cut using a band saw.

Results

The piglet's thoracic spinal column consisted of fourteen thoracic vertebrae. Based on the CT examination of the vertebral column, an expressed lordosis was observed between the sixth thoracic (Th6) and the first lumbal (L1) vertebrae. The lordosis was distinct between the Th9-Th10 vertebrae. Due to the abnormality of lordosis, the shape of the rib cage was anatomically irregular. The ribs had an expressed curved body (from the costal tubercule till the upper third of the body of the rib) in the caudal direction starting from the third rib. All vertebral bodies in the thoracic and lumbal region were physiological and no malformations were detected. All articular facets (cranial and caudal articular processes) and the spinous processes were normal.
On the basis of the T2 weighted images acquired during the MR examination of the vertebral column, no abnormalities were found except for a hypointensity of the Th3-Th4 intervertebral disc (loss of water content).
Additionally, hypoplasia of the ventral nasal conchae and the ethmoturbinalias was detected on both sides by CT scans.
MRI and CT scans highlighted the same results as it was revealed at necropsy.

Discussion & Conclusions

This is the first CT/MR imaging of juvenile kyphosis. CT/MRI fusion provides a proper technique for scientific study of the previous and early stage as well as the clinical course of kyphosis in pigs.

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ANTIMICROBIALS IN FEED OR WATER FOR PIGS IN SWEDEN IN A PERSPECTIVE OF 30 YEARS

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Introduction

In 1986, the use of antimicrobials as feed additives for growth promotion was stopped in Sweden. Before 1986, practically all piglets were fed antimicrobials from weaning until the age of 10-12 weeks. The aim of this study was to explore what happened with the use of antimicrobials for group medication in a long term perspective, and relate this to current figures on antimicrobial resistance in the commensal flora of pigs.

Material & Methods

Data on use of antimicrobials and on resistance in bacteria isolated from animals are presented annually in the Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM; **1**). Statistics on sales of feed with zinc oxide is available from the Board of Agriculture.

Results

Before the ban in 1986, the average total sales of antimicrobials mixed in feed or water was 30 tons of which about half was for growth promotion.

From 1988, the use of medicated feed or water for animal species other than pigs has been insignificant. At that time the usage of in particularly olaquindox and tetracyclines on veterinary prescription increased, and the total sales averaged 15 tons from 1988 to 1993. In the following years, a decrease was noted. Initially, this was explained by a common use of zinc oxide, but since 1998, the use of zinc oxide is subject to veterinary prescription and is since then used by less than 10% of the herds (**2**). In 2008, the sales of antimicrobials for group medication were 9% of the average of the sales before 1986.

The prevalence of resistance among bacteria from Swedish animals is favorable when compared to materials collected and tested in similar ways in other European countries (**1**, **3**).

Discussion & Conclusions

In a long term perspective, the ban of growth promoting antimicrobials has been followed by a considerable decrease in usage of antimicrobials for group medication. There have been no negative consequences for the prevalence of resistance among food borne pathogens or commensal bacteria.

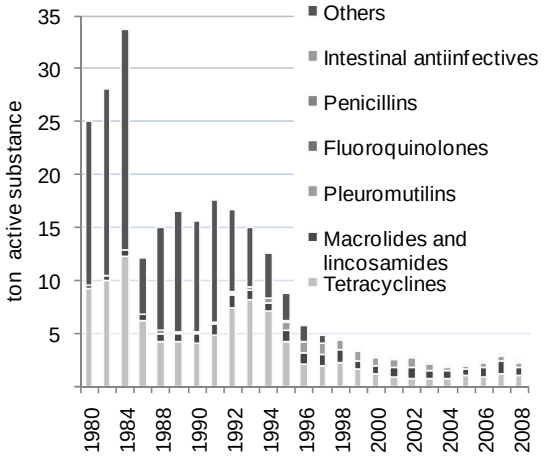

Figure 1. Sales of antimicrobials for growth promotion or in feed or water medication in Sweden. "Others" represent substances no longer available.

Initially, there was an increased need for veterinary prescription of products for group medication, which triggered a number of interventions aiming to improve animal health. Guidelines on veterinary prescription for group medication were developed, and a herd specific plan for disease prevention by other means was a mandatory part of that policy.

Buildings and systems for management, including feed composition, hygiene and a control of the flow of animals through the production were improved generally. Also control programmes for specific diseases were important, as the concept of biosecurity was introduced in participating farms.

The Swedish experience shows that it is possible to considerably reduce the need for antimicrobials in pig production, and that this is favorable from an antimicrobial resistance point of view. To achieve that, a combination factors including engaged farmers and veterinarians, extension services and active problem oriented research is needed.

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EFFECTS OF DISEASE ON BEHAVIOURAL, CLINICAL AND CLINICAL-CHEMICAL INDICES IN PIGS

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Introduction

Absence of disease has been given highest priority in the assessment of quality of life of farm animals (e.g. Dawkins 2006). However, a disease-free state cannot always be ensured. Thus, infectious diseases are major causes for pain and suffering (Hart 1988, Algers, 2004). During infection, sickness and suffering refer to a coordinated set of behavioural and physiological changes, leading to the concept of sickness behaviour. Behavioural indices are rarely assessed in conjunction with clinical diagnostics in more than a qualitative manner. Knowledge of the effects of model diseases on behaviour and on relationships among clinical/clinical-chemical parameters and behavioural indices would permit a better understanding and assessment of animal suffering. The aim of this study was to describe physiological and behavioural patterns and relationships in pigs before, and during the acute, subclinical and chronic stages of a model disease.

Material & Methods

Based on a well defined model disease (*Sarcocystis miescheriana*), we have studied behavioural patterns (lying inactive [LYWA], activity during lying [ADLY], feeding [FEED], drinking [DRNK], rooting [ROOT], walking [WALK], and social interactions [SOCB] of pigs during stages of health (day 0, before infection), acute disease (day 14 post infection [p.i.]), subclinical disease (day 28 p.i.), and chronic disease (day 42 p.i.). Data were captured from video records of 139 F_2 Meishan x Pietrain crossbred pigs between 09:30 a.m. to 11:30 a.m. The time interval for observation was chosen so as to overlap with the first activity peak in the morning and to avoid times of human intervention. Behavioural indices were correlated with a set of clinical-chemical parameters (red and white blood cells, serum enzymes, serum metabolites and electrolytes).

Results

Before infection, respective time fractions were 56% (LYWA), 21% (ADLY), 10% (FEED), 0.9% (DRNK), 6.3% (ROOT), 2% (WALK), and <1% (SOCB). This behavioural pattern changed distinctly during disease. Overall activities were reduced from 44% (day 0) to 10% (day 14 p.i.), 34% (day 28 p.i.), and 20% (day 42 p.i.).

The development of different stages of disease was accompanied with distinct changes in clinical and clinical-chemical parameters. Deviation from the population´s "standard" activity (before infection) could be predicted with clinical-chemical parameters (e.g. alkaline phosphatase [AP]).

Behavioural activities were generally reduced, whenever clinical/clinical-chemical traits changed to pathological values. A multivariate regression model revealed five predictors to explain e.g. total activity. The model was statistically significant with $P < 0.0001$ and explained 32% of the pigs' total variance for this indicator (correlation coefficient 0.564). AP was the most important predictor. A decrease of 100 IU/l was associated with a reduction in activity of 7.8% during the observation period. A 10% reduction in leukocytes or 10 mmol/l less serum sodium ions were each equivalent to a more than 5% increase in activity. Increases of 10 µmol/l in serum creatinine or 1000 IU/l in serum creatine kinase (CK) were associated with a decrease in activity by another 1.4 and 5.1%, respectively.

Discussion & Conclusions

To date, most welfare research has been concerned with identifying effects of housing conditions on welfare. The present study substantiates the role of infectious diseases in animal welfare. Taking into account the high prevalence of infectious diseases, it surprises that there is still very little research published on how different common diseases affect an animals' welfare (Algers, 2004). With more information about effects of defined model diseases on behaviour and on relationships among easily obtainable clinical/clinical-chemical parameters and behavioural indices, our understanding and assessment (eventually even under field conditions) of animal suffering due to infectious disease, even under subclinical conditions, might be decisively improved.

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PRELIMINARY RESULTS ON THE LINK BETWEEN BIOSECURITY STATUS AND HERD CHARACTERISTICS, DAILY WEIGHT GAIN AND MORTALITY

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Introduction

It is believed biosecurity influences production results, nevertheless, few studies succeed in demonstrating this link. A biosecurity scoring system was used to study the relation between the biosecurity on a farm and herd characteristics, daily weight gain and mortality.

Material & Methods

Thirty one randomly selected Belgian pig herds were visited. The herds had at least 80 sows and 500 fattening pigs. During a herd visit, the biosecurity status of the herd was quantified by means of a biosecurity scoring system (Laanen et al., 2010). At the same time additional data concerning the herd and production characteristics (number of sows, number of piglets, number of fattening pigs, age of the buildings, years of experience of the farmer, daily weight gain and mortality of fattening pigs) were collected.

Results

Herd characteristics:

A positive correlation was found between the number of sows on a herd and both the external ($r = 0.43$, $p = 0.02$) and internal ($r = 0.34$, $p = 0.06$) biosecurity. Also the number of piglets (< 25kg) and the external biosecurity were positively correlated ($r = 0.39$, $p = 0.03$). The correlation with the internal biosecurity was only weakly positive ($r = 0.19$, $p = 0.30$). No correlation was found between the number of fattening pigs on a herd and the external or internal biosecurity. Both the age of the buildings and the years of experience of the farmer were negatively correlated with the internal ($r = -0.41$, $p = 0.02$; $r = -0.21$, $p = 0.27$ respectively) and external ($r = -0.14$, $p = 0.46$; $r = -0.09$, $p = 0.64$ respectively) biosecurity.

Performance of fattening pigs:

The daily weight gain of the fattening pigs was positively correlated with the external biosecurity ($r = 0.28$, $p = 0.15$), but no effect of the internal biosecurity ($r = 0.03$, $p = 0.86$) on the daily weight gain was found. No clear effects of the biosecurity status of the herd on the mortality of the fattening pigs were found.

Discussion & Conclusions

These results clearly indicate the existence of a positive correlation between the number of sows or piglets on a herd and the level of biosecurity, indicating that farmers with larger herds pay more attention to biosecurity than those with smaller herds. The larger a herd becomes, it is more likely to be more professional and well managed. No comparable effect was seen for the number of fattening pigs on the herd. It should also be noticed that the found correlations are moderate to low.

It is also noticeable that the biosecurity increases with a decreasing age of the buildings. This illustrates that in more modern infrastructure more attention is paid to biosecurity. The same holds for the years of experience of the farmer suggesting that younger farmers are more interested in, and willing to apply biosecurity measures.

When evaluating the link with the daily weight gain of the fattening pigs it appears that this increases with an increasing external biosecurity, whereas for mortality no clear link could be identified.

Several of the found correlations are not statistically significant and therefore need to be interpreted with care. Yet in this study only the results of the first 31 herds out of an ongoing study on 100 herds are presented. Therefore it is believed that with increasing sample size several of the observed trends will become statistically significant. Moreover, the data will also be further explored to identify the parts of biosecurity that are the most influential on health and production parameters.

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THE USE OF AN ONLINE SCORING SYSTEM FOR THE QUANTIFICATION OF THE BIOSECURITY STATUS IN PIG HERD

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Introduction

In order to quantify the biosecurity situation on pig farms a biosecurity scoring system was developed by the Veterinary Epidemiology Unit of the faculty of Veterinary Medicine, Ghent University and incorporated in a free online application (www.biocheck.ugent.be).

Material & Methods

The scoring system takes both external and internal biosecurity measures into account. Both parts are divided into 6 subcategories each consisting of 2 to 13 questions. Each subcategory received a weight based on literature on pathogen transmission and general knowledge of infection risks. A score between 0 and 100 is obtained for both external and internal biosecurity. The scoring system is adapted to be appropriate for every type of pig unit (fattening herd, breeding herd, mixed herd, etc).

Results and discussion

The scoring system has been available for pig farmers in Belgium for one year and 138 herds (i.e. 13 breeding herds, 6 fattening herds and 119 mixed herds) have filled in the questionnaire until January 2010. The average score for external biosecurity is 64 (min 29; max 95). The score for internal biosecurity is lower in most farms (91%) with an average of 51 (min 18; max 89). There is a strong positive correlation (r=0.63) between the scores for external and internal biosecurity.

Some selected results relating to external biosecurity show that 81% of the herds purchasing new breeding animals use quarantine facilities for an average period of 36 days. 96% provides farm-specific clothing and footwear to visitors, but only 29% ask visitors to wash and disinfect their hands. Nevertheless, only in 58% of the herds, the farmer and personnel also carry out these hygienic measures themselves before entering the stables.

Concerning internal biosecurity, only 38% of the farmers house diseased animals in separate hospital pens and 48% manipulates the diseased animals after the healthy ones. Suckling piglets are transferred between sows on 98% of the herds, of which 43% performs this operation after 4 days post partum. All-in all-out management is practiced in 82% of the herds in the nursery unit, 70% of the herds in the fattening unit. Nevertheless, 21% puts smaller but older pigs together with the younger ones in the nursery and/or fattening unit. From all the herds, 62% cleans and disinfects every stable after a production round, but only very few (5) verifies the efficiency of these measures. 54% always applies a sanitary stand empty period after each production round.

The large variation in scores of different farms shows that there is room for improvement in many participating herds. On average, the scores for external biosecurity, which are mainly measures related to infrastructure or measures imposable on other parties, are higher than the scores on internal biosecurity which are more related to the work and management strategies of the farmers themselves. As the results show, there are many biosecurity measures that have become common practice for farmers in Belgium; on the other hand, some effective biosecurity measures should be more frequently practiced. It needs to be emphasized that these 138 farms voluntarily participated in this study and may therefore not be representative for all pig herds. Therefore it is likely that the general results are lower than the results presented here.

The biosecurity scoring system is used in another study (Laanen et al., 2010) to relate biosecurity in a quantitative way with health and production parameters.

References

- Laanen et al. (2010). Preliminary results on the link between biosecurity status and herd characteristics, daily weight gain and mortality (Poster abstract submitted to ESPHM 2010).

COUGH SOUND ACOUSTICS ENVIRONMENTAL AGENTS IN MODERN SWINE FARMING.

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Introduction

In intensive swine farming conditions respiratory disease outbreaks are often linked to housing conditions, environmental predisposing factors and etiologic agents. All these actors make it a multifactor diseases that require punctual observation both on the sanitary and environmental levels of the herd. In nowadays the extremely high number of animals in farms is associated with an increase of diffusible pathologies and with a more difficult controlled environment. A continuous observation of animals health and its relation with environment and abiotic disease causes, can't be quantified by humans. We propose in this paper how continuous cough sound analysis might allow automatic detection of respiratory disease where coughs are markers of infection. Coughs sounds might also link with climate parameters, particulate matter and ammonia concentrations.

Material & Methods

Acoustic and environmental data were collected in a piggery during seven months of a whole fattening cycle (30-175 kg) in an Italian piggery. Microphones were hanged over the pens and the recordings were run 30 minutes for cough sounds collection. The data were stored via Adobe Audition to a pc unit. Particulate matter concentrations (PM_{10}) were monitored by a sampler (Haz Dust-Epam 5000) that allows measurements in mgm^{-3}. The device was located inside the building in such a way that the air velocity was in general less than 0.5 ms^{-1}. For the ammonia sampling the portable Dräger Chip Measurement System was used for spot gas measurements. Spot temperature and relative humidity inside the piggery were assessed by a portable data logger.
All the samplings were collected once a week. Sound playbacks were than labeled and sound analysis was than done on single coughs to investigate their acoustics parameters. The data were submitted to variance analysis (Proc GLM, Sas institute) and to Pearson correlation to estimate the effects of environmental conditions on respiratory disease in pigs.

Results

Coughs were extracted from the collected audio files and their duration, amplitude and frequency were analyzed.
From the statistic analysis, over 4204 datasets were collected during the 7 months. The number of coughs collected every 30 minutes was positively influenced by the grouping of relatively acceptable environmental parameters: animals showed a higher number of coughs attacks every 30 min when dust concentration was lower than 59 micrograms and ammonia ranged from 10 to 20 ppm. Pearson correlation showed that increase of animals' age corresponds to increase of sound duration (19 %, $P<0.001$), decrease of the peak frequency, fundamental frequency and of the number of coughs recorded in 30 min. Dust concentration increase corresponds to a decrease in number of coughs recorded in 30 min (-26 %, $P<0.001$). The higher Nh_3 level lead to shorter sound duration, increase in amplitude and fundamental frequency (resp.14 %, $P<0.01$ and16%, $P< 0.05$) and decrease in the number of coughs recorded in 30 min (-24 %; $P< 0.001$).

Discussion & Conclusions

Sound acoustic characteristics can be influenced by environmental factors in different ways: dust, ammonia levels and HR act on the respiratory system modifying the structures involved in cough generation.
Peak and fundamental frequencies were in this case affected by high dust concentration and low HR, the diminution of these parameters is possibly due to airways obstruction due to mucus production. The effect of rare lung clearance capacity due ammonia exposure is confirmed by the decrease of the number of coughs and from their shorter duration indicating reaction failure of the respiratory system. The findings within this study demonstrate how cough sounds may be variable according to the respiratory system dynamics influenced by several agents. So we can assume that monitoring acoustics may provide continuous observation of the health status of animals and knowledge of the a biotic factors involved in a respiratory pathology.

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INSPECTION METHODS TOWARDS MORE SUSTAINABILITY IN PIG PRODUCTION

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Introduction

In herd health and production management programmes it is common use to make an inventory of the herd performance status. The activities comprised under "inventory" are often called "monitoring". This is an important component of quality risk management programmes following combined HACCP and FMEA concept as well (Schmitz 2005). Monitoring is an act of conducting a planned sequence of observations or measurements of certain control parameters to assess whether a certain point in the production process is under control or functioning correctly or shows conformity with market or society demands. It is highly indicated to conduct also an inventory regarding the prevailing risk conditions on the farm for animals, their environment, the management and the farm records (Mack et al. 2006).

Material & Methods

A literature review has been performed to investigate weak points to sustainability at farm level. Discussions with experts for each sustainability criteria and an inventory of possible inspection methods for the sustainability criteria were combined to create a reference model for an inspection strategy towards more sustainability. Preventive quality management methods have been tested to support decision making processes, to assure conformity to demands and to increase income.

Results

A selection of sustainability criteria which need a continuous inspection on farm level has been performed. As most of the criteria were rather fix or changing only on the long-term, the most flexible ones have been investigated. Animal health, welfare, meat quality and safety were selected, as they were directly influenced by decisions in the operative business. To these criteria a monitoring of conformity or nonconformity during the production can gain additional information. Six important inspection points have been investigated in the fattening process of pigs (fig. 1). This model is designed to detect the most relevant disturbing factors affecting the growth of a pig. The inspections are focusing on:

- health and performance of the pigs or groups of pigs
- environment of pigs
- farm management
- process information
- transport and slaughter

Quality backward and forward chains show the importance of the information flow in both directions of the production process. Data investigated on one step often can support decisions on another step of the production (Petersen et a. 2008).

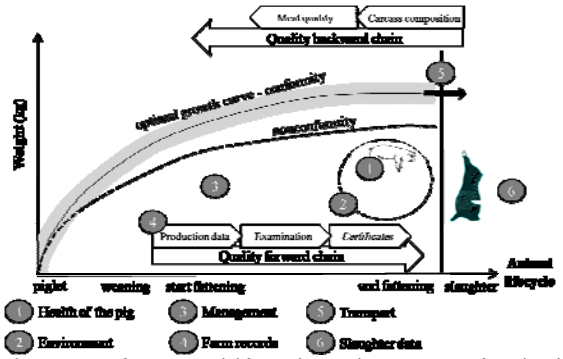

Fig. 1: Reference model for an inspection strategy at farm level

Possible relevant inspection methods have been collected and they will be assessed by demands for practical use.

Discussion & Conclusions

The awareness of the direct linkage of all sustainability criteria is increasing in the public opinion as well as in legislation. A risk based inspection method to identify and reduce weak points for on farm sustainability will present a great benefit for farms confronted with future challenges.

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C-REACTIVE-PROTEIN, A USEFFUL BIOMARKER FOR HERD HEALTH MONITORING?

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Introduction

Acute-phase-proteins such as Haptoglobin and C-Reactive-Protein (CRP) are proposed recently for monitoring herd health status or for controlling efficiency of antimicrobial metaphylaxis or immunoprophylaxis. Particularly CRP as a specific biomarker for bacterial infections demonstrated a multiple increase in serum concentrations within a few hours following experimental infection with *H. parasuis* (4), *S. suis* (5) and *A. pleuro-pneumonia* (APP), (1, 2, 3).

Against this background CRP determinations were part of a clinical study that was originally aimed to compare the efficiency and profitability of two different metaphylactic regimens in weaned pigs naturally exposed to APP as a persistant heard health problem.

Material & Methods

A total of 348 piglets of one farrowing series was weaned with 21 days and separated to antimicrobial metaphylaxis with Tulathromycin by a single dose injection or with Amoxicillin by oral feed medication lasting 7 days, and a non medicated control. For feed-technical and space reasons controls and Tulathromycin medicated weaners had to be housed in the same pens.

For CRP determinations an independent sample of 20 weaners in metaphylaxis groups, each, and a sample of 15 weaners in the control group were bleeded at weaning (day 0 before separation), at day 21, 35 and 49 after weaning, each. Analysis was performed by a commercial laboratory (*biocheck*, Germany) using the Porcine-C-Reactive-Protein Assay (*tri-delta*, Ireland).

Results

Both metaphylactic regimens prevented respiratory diseases and losses during their pharmacologically effective period. Even controls were not diseased during this time. However, later on, at the beginning of the 7th week 94% of initially Amoxicillin medicated weaners went down with an acute, febrile pleuropneumonia (APP) which could be treated successfully with oral feed medication using Amoxicillin again.

At weaning (day 0) the CRP-median was 87 µg/ml, the reference range (2.5-97.5 percentile) was 7 to 1184 µg/ml. At the follow-up dates the medians of the control group were 4-5times higher ($p < 0.01$), the medians of the Tulathromycin metaphylaxis were even 5-10times higher ($p < 0.001$) without any enzootic disease, the medians of the Amoxicillin metaphylaxis were 3 times higher only on day 21 and 49. On day 35, concurring with the period of incubation before clinical manifestation of the APP-infection one week later, the CRP-median was almost equal to day 0, but significantly lower ($p < 0.001$) compared to the other groups on day 35. Weaners with Tulathromycin metaphylaxis exceeded more often the day 0-upper reference limit (>1184 µg/ml) over time. On day 21 this difference was significant with 31 vs. 0% ($p < 0.05$).

Discussion & Conclusions

Compared to Amoxicillin the lasting effect of Tulathromycin with regard to APP-protection may be due to the combination of bacteriostatic and bactericidal properties, and a longer pharmacologically effective period. Controls in the same pens seemed to benefit from the effective germ reduction, too. However, a relatively high CRP-level and a lack of APP-infection in both groups were incompatible with other studies. With respect to the low level at weaning this might be interpreted as a sign for a non disturbed development in active immunity. The low level at time of natural infection (incubation) in the Amoxicillin group was also in contrast to high levels following experimental infections in other studies. This might be a result of ongoing germ exposition since weaning by a less efficient metaphylaxis. However, these controversial findings and their hypothesis should be clarified in further field studies on the basis of bigger sample sizes, shorter sampling intervals and more convenient sampling media (e. g. saliva), the latter particularly for suitability in practice.

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KEY REPRODUCTION PERFORMANCE PARAMETERS IN ANCIENT EUROPEAN BREEDS (IBERIAN PIGS)

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Introduction

Technical efficiency and control of the production cost is more important than ever in European pig production. This is so for classical swine production but is becoming critical as well for other breeds, with very different physiological characteristics. This paper is aimed to provide a first structured batch of the most important reproductive parameters of a very different pig breed (Iberian pig) that has been able to develop a successful industry of high quality products around (fresh pork and cured products).

Material & Methods

A total of 8 farms and 3400 sows providing data in a routine were included in this first study with the summary of 2009 results. Data were imported from different software brands, uniformized and merged in a single data base, from which the analysis was performed. Overall approach was based in the differential diagnosis proposed by Dial et al (1992) and the split of Non Productive Days (NPD) was base in the six components proposed by Koketsu (2005).

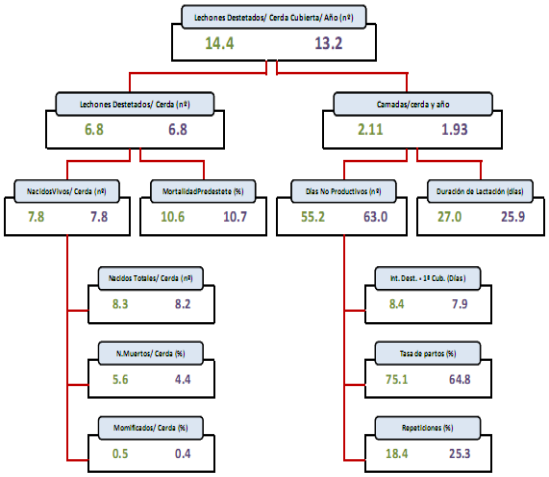
Results

Reproductive performance of this pig breed is much lower than in other modern pig lines (Chart 1). Piglets weaned / sow / year (W/S/Y) were 14.4 explained both for a short number of weaned per sow (6.8) and a low number of farrowings per year (2.11). Number of piglets born in total is low as well (8.3) but pre-weaning mortality is not particularly low (10.6 %). The lactation length is of 27 d with a number of NPD rather high (55). Finally, weaning to oestrus interval is higher than in modern breeds (8.4 d). Farrowing rate is lower than modern breeds standards (75.0 %) with a higher percentage of sows repeated to oestrus (18.4 %).

Regarding specific phases of the production, must be highlighted the following facts:

- Replacement gilts were included in a lower percentage to the needs.
- Repeated to oestrus were high because of a deviation in cyclical ones (both first and second), late repetitions and empty sows.
- Prolificacy curves show a similar pattern compared to modern breeds
- PWM is unusually high from week 2 onwards (14.7 % of total losses)

Chart 1. Productivity tree in Iberian Pigs.



NPD days are in general high and its distribution shows some interesting facts:

- Input due to return to oestrus (38 d average)
- Abortions tends to be rather late (79.7) as well as deaths (74.7)
- Waiting time at farm for culling is high (46.7)
- Weaning to oestrus interval is higher than in other breeds (8.4 d).

Discussion & Conclusions

These results shows an overall different reproductive performance of other breeds that can be explained both for their physiological differences and the kind and quality of the management used with them. The knowledge derived from this factors can be used for the prioritization of the problems found and the later implementations of the solutions at farm scale. On the other side, performance of other European local breeds can analysed following the same pattern in order to stablish a harmonised set of data.

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EFFECT OF ALTRENOGEST ON PREVENTION OF EARLY PARTURITION AND REPRODUCTIVE PERFORMANCE IN SOWS

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Objectives

The purpose of this study was to investigate the efficacy of altrenogest administration during late pregnancy to prevent early parturition (< 114 days of gestation) and the effect of this treatment on the reproductive performance in sows.

Material and Methods

The experiment was conducted in 4 commercial pig herds located in Flanders, Belgium. Within each herd, random stratification based on parity was used to allocate the sows (n=329) into a treatment or control group. Oral administration of 20 mg of altrenogest (Regumate®, Janssen Animal Health) was performed on day 111, 112 and 113 of gestation, based on the sow's individual insemination date. Reproductive parameters recorded were: farrowing date, number of live born piglets, stillborn piglets, mortality of weak born piglets during the first 3 days postpartum, cross-fostered piglets, pre-weaning mortality, number of weaned piglets. Statistical analysis was performed by means of analysis of variance taking herd into account as a random factor (SPSS 16.0).

Results

Early parturition was found in 14.1% of the control sows. The reproductive performance of sows with an early parturition (C1) was significantly lower compared to sows with a normal gestation length (≥114d, C2) and compared to sows treated with altrenogest (T) (Table 1).

Gestation length was significantly different between the treatment group and the total control group (C1 + C2) (115.3 ± 1.23 and 114.7 ± 1.69 days, respectively $P < 0.01$). In the treatment group, none of the sows started farrowing during the altrenogest treatment. For the other reproductive parameters, no significant differences were found between the treatment group and the total control group (C1 + C2) and between the treatment group and the control group with a normal gestation length (C2) ($P > 0.05$).

Table 1: Reproductive performance (mean (s.d.)) of the sows in the treatment group (T) and the control sows either with early parturition (< 114 days) (C1) or with a normal gestation length (≥ 114 days) (C2).

Parameter	C1 n= 23	C2 n= 140	T n= 166
Gestation length (Days)	111.9 (1.4)[a]	115.2 (1.2)[b]	115.3 (1.2)[b]
Total litter size (No)	14.9 (4.9)	14.2 (3.9)	14.4 (3.9)
Live born piglets (No)	11.6 (4.2)	13.0 (3.7)	12.8 (3.7)
Stillborn piglets (No)	3.4 (3.4)[a]	1.2 (1.4)[b]	1.6 (1.9)[b]
Pre-weaning mortality (No)*	2.7 (2.7)	1.7 (1.7)	1.8 (1.9)
Mortality of weakborn piglets (%)*	20.7 (31.2)[a]	5.9 (8.9)[b]	6.4 (15.8)[b]
Weaned piglets (No)*	8.1 (5.0)[a]	10.7 (2.1)[b]	10.5 (2.9)[b]

Within a row, significant differences are marked with different superscripts ($P<0.01$)/ * Sows with a correct record of cross-fostered piglets and pre-weaning mortality (n= 17, 91 and 130 for C1, C2 and T, respectively)

Discussion and conclusion

The present study demonstrates that early parturition adversely affects the reproductive performance of the sows. The administration of altrenogest at D111, 112 and D113 of gestation is an effective and safe method to prevent early parturition and can counteract the reproductive losses due to premature farrowing. However, it does not significantly influence the reproductive performance parameters compared to non-treated sows with a normal gestation length.

EFFECT OF EARLY PARTURITION ON THE REPRODUCTIVE PERFORMANCE IN SOWS

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Objectives

The purpose of this study was to determine the incidence of early parturition (< 114 days of gestation) and to investigate the effect of early parturition on the reproductive performance in sows.

Material and Methods

This study was based on reproductive data records (Cercosoft NV, Oudenaarde, Belgium) of 35 herds containing 87,978 farrowing records of which 26,988 records were excluded because induction of parturition was performed or due to missing information on farrowing data. Hence, 60,990 farrowing records of 17,401 sows were used for further analyses. The association of the outcome variables (total litter size, live born piglets, stillborn piglets) with the gestation length (GL) was assessed using a multilevel linear mixed effect model, with herd included as random factor, and parity and breed as fixed factors. The influence of the duration of the previous gestation on the next was also determined. All statistical analyses were performed in MLwiN 2.0.

Results

The mean gestation length was 115.4 ±1.62 days. Early parturition occurred in 10% of all farrowing events (Fig 1).

Fig 1: Frequency distribution (%) of gestation length in 60,990 farrowing records.

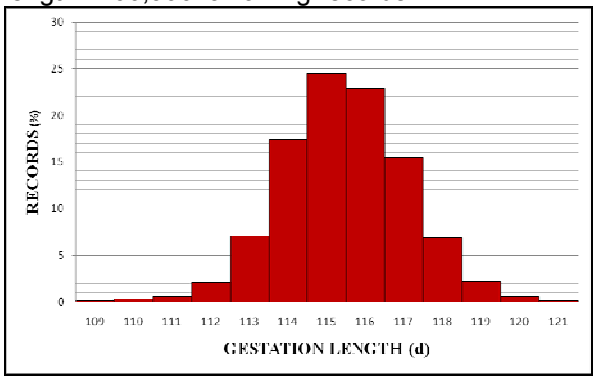

Table 1 shows that sows with GL <112d have more stillborn piglets and less piglets born alive compared to those with GL 114-117d. A GL of 112-113d was associated with more total born piglets, more piglets born alive and more stillborn piglets. Sows with GL ≥ 118d were more likely to have less total born piglets and less piglets born alive compared to those with GL 114-117d. Sows with a gestation length of < 114d were more likely to have a gestation length of < 114d at the subsequent parity (OR: 1.2) as those with a gestation length of ≥ 114d (P<0.05).

Tabel 1: Mean (s.d.) of total litter size, live born and stillborn piglets by gestation length (GL)

GL (d)	n	Total litter size	Live born piglets	Stillborn piglets
109-111	576	13.1 (3.7)	**10.5 (4.4)**	**2.6 (3.4)**
112-113	5544	13.8 (3.3)	**12.5 (3.2)**	**1.3 (1.8)**
114-117 = ref.	48929	13.3 (3.5)	12.4 (3.3)	0.9 (1.6)
118-121	5941	**11.9 (3.9)**	**10.9 (3.8)**	1.0 (1.7)

Within a column, values different from the reference are marked in bold (P<0.05)

Discussion and conclusion

The present study showed that early parturition (< 114d) occurred in 10% of the farrowing events. Early parturition significantly increased the risk for stillborn piglets, especially in sows with a GL < 112d. This is likely due to the relative immaturity of the piglets at parturition (1). Large litters and more piglets born alive were associated with a GL < 114d, while small litter size and low numbers of live born piglets were related to a GL ≥ 118d. This is in agreement with Sasaki and Koketsu (2007). Sows with a short gestation length were more likely to have an early parturition at the subsequent parity. This information may be important for producers to identify potential sows-at-risk. It may enable them to improve the reproductive performance by assisting farrowing or by preventing an early parturition.

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COMPARISON OF PRODUCTIVITY BETWEEN PRIMIPAROUS AND MULTIPAROUS SOWS PROGENIES IN A LOW-HEALTH STATUS FARM

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Introduction

Primiparous sows progenies (PRIM) show lower body weight (BW) at birth and higher pre-weaning mortality, affecting later productive performance in comparison with multiparous sows progenies (MULT) (1,2). The reason is unknown but might be related to the lower immune transmission via colostrum, resulting in higher susceptibility to pathogens. Therefore, it is expected that differences between PRIM and MULT pigs were higher in low-health status conditions. The aim of this study was to know the impact of PRIM on later productive phases, in a low-health status farm with *A. pleuropneumoniae*, *M.hyopneumoniae*, *P. multocida*, PRRS and PCV2 confirmed positive diagnosis.

Material & Methods

The experiment was performed in a farrow to finish farm with 700 sows to assess the parity effect on average daily gain (ADG) and susceptibility to pathologies. A total of 300 pigs, 150 PRIM and 150 MULT, were followed from birth to slaughter. Individual ADG from 0 to 151 days of age, incidence of clinical signs and lung scoring at slaughtering at 100 kg BW, were controlled. Productive performance data were analysed by the GLM procedure of SAS (v 9.0) for randomised complete block designs. Mortality and lung scoring data were analysed by the CATMOD procedure of SAS.

Results

Average productive values were poor (540 g/d ADG; 14.5% mortality), confirming low-health status of that herd. PRIM pigs showed worse ADG in all productive phases (P<0.001; table 1), resulting in lower final BW than MULT pigs (78.9 vs 89.0 kg; P=0.0001).

Table 1. Mean average daily gain (g/d) results.

	Lactation	Nursery	Fattening	Total
MULT	245	412	628	574
PRIM	209	358	556	507
SEM[1]	*8.44*	*13.40*	*14.46*	*12.76*

[1]Standard Error of Mean

Mortality rates of MULT and PRIM pigs are presented in table 2.

Table 2. Mortality rates

	Lactation	Nursery	Fattening	Total
MULT	2.17	3.81	5.61	11.18
PRIM	0	3.23	15.00	17.74
SEM[1]	*NS*	*NS*	*	*

*Probability; NS, P>0.05; *, P<0.05

Total mortality rate was higher in PRIM than in MULT pigs (P<0.05), but not in all phases. In lactation, the main causes of mortality were crushing by sows and starvation, and no differences were observed between treatments. In nursery main cause of mortality was diarrhoea due to beta-haemolytic E. coli. This pathogen affected equally both MULT and PRIM piglets. Finally respiratory pathologies in the fattening period significantly affected more PRIM than MULT pigs (P<0.05), confirming PRIM have higher susceptibility to respiratory pathologies. At the abattoir, a high percentage of lung with pneumonia lesions (38%) were observed, confirming the high pressure of respiratory disease in the experimental herd. Between sow origin, no significant difference in the lung scoring were observed (40.4% vs 36.3% in PRIM and MULT pigs, respectively).

Discussion & Conclusions

As a result, economical returns over investment (ROI) were lower in PRIM than in MULT pigs. Based on market prices of 2009, when the experiment was run, 12.8 € more per pig produced were obtained for MULT for PRIM pigs (P<0.001).

We conclude that PRIM progenies show lower productive performance and higher pathologies prevalence. These differences seem to be higher in low-health status conditions, especially in the fattening period. Rearing separately primiparous sows progeny might be a good management practise for commercial farms.

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HEALTH BONUS AFTER TRANSITION TO 4- OR 5- WEEK BATCH MANAGEMENT SYSTEMS

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Introduction

Sow batch management systems (BMS) become more popular because of advantages in labour planning, batch size of piglets, strict all-in all-out practices and health management. The 4- and 5-week BMS have advantages compared to a 3-week BMS, especially with regard to general health (1). Weaning piglets at 3 weeks of age in large batches creates possibilities for a significant improvement of the general farm health status. However, exact data on a health bonus with these management systems are not available. Therefore, a field study (project 'Veepeiler-varken' funded by Sanitary Fund) was designed to measure the health bonus for pig farms when changing from a conventional 1-week management system to a 4- or 5-week group management.

Materials and Methods

Ten Flemish wean-to-finish pig farms, changing from a conventional 1-week management system to a 4- or 5-week BMS were included in the trial. The average farm size was 227 sows, with a minimum of 130 and a maximum of 500. Samples were collected before and 24 months after finishing transition to the BMS. The type of samples and the different analysis are described in Table 1.

Table 1. Sampling schedule before and 2 years after transition

Sample type	Animal category	Type of analysis**	# of samples
Blood/serum	Gilts	PIA, PRRSv, M. hyo	10
	Sows (> 3 litters)	M. hyo	10
	Piglets (10 wk)	PIA, PRRSv, App	10
	Grower pigs (14wk)	PIA, PRRSv, App, M.hyo	10
	Finisher pigs (> 80 kg)	PIA, PRRSv, App, M.hyo	10
Nasal swabs	Piglets/growers (6-10-14 wk)	PCR DNT*	3 * 12
Mixed faeces (faeces of 5 animals)	Growers/finishers (14 & 20 wk)	PCR *Brachyspira* spp.	2

* DNT = *Pasteurella multocida* dermonecrotic toxine

** ELISA test on serum samples

The following ELISA-tests were used: PIA – Bioscreen Enterisol® Ileitis ELISA, PRRS – Idexx HerdChek PRRSv Ab test, *M. hyopneumoniae* – Idexx HerdChek M. hyopn. Ab testkit and *A. pleuropneumoniae* – Idexx Chekit – App – ApxIV ELISA.

Results

The results of the serological screening are presented in Table 2. The nasal swabs were all negative for *Pasteurella multocida* DNT and no clinical signs of atrophic rhinitis could be observed. All faecal samples were negative for *Brachyspira hyodysenteriae*. Nevertheless, minor pathogenic species of *Brachyspira* were detected, both before and after the transition.

Table 2. Serological results, expressed as % positive animals before and after transition to a 4- or 5-week BMS and % change before-after

Disease	Animal group	Before	After	% change
PIA	Gilts	90	90	0%
	Piglets	22	5	**-77%**
	Grower pigs	52	30	**-38%**
	Finisher pigs	64	70	-9%
PRRSv	Gilts	90	83	**-8%**
	Piglets	54	61	+13%
	Grower pigs	80	72	**-10%**
	Finisher pigs	80	89	+11%
M. hyo.	Gilts	51	49	**-4%**
	Sows	16	36	+125%
	Grower pigs	51	32	**-37%**
	Finisher pigs	71	46	**-40%**
A. pleuropn.	Piglets	55	65	+18%
	Grower pigs	40	35	**-13%**
	Finisher pigs	74	51	**-31%**

Discussion and conclusions

Transition to BMS seems to postpone or slow down the seroconversion for PIA, M. hyo and App. This allows the farmer to vaccinate the pigs before they come in contact with the pathogen. It also allows more time for the pigs to build up immunity. The speed and level of horizontal transmission within the batch is determined by weaning age, maternal protection and transmission of pathogens from the sows to the piglets. The degree of separation of the batches from each other then determines the vertical transmission between the batches. In conclusion, the results show a clear health bonus for pig herds with transition to a 4- or 5-week BMS.

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A DESCRIPTIVE QUESTIONNAIRE ON WEANING MANAGEMENT IN COMMERCIAL PIG HERDS IN BELGIUM

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Introduction and objectives

Suboptimal reproduction results in modern pig herds are not only disease related. Many different non-infectious factors such as length of lactation, body condition, season, and weaning management practices have an influence on the fertility of sows (1). The purpose of this epidemiological study was to investigate how weaning management influences different reproduction parameters in commercial pig herds in Belgium.

Materials and Methods

In August 2009, a questionnaire was sent by conventional mail to 150 randomly selected pig herds in Flanders, Belgium, with exclusion of farms smaller than 80 sows. After one and a half months, all farms were contacted by phone and asked whether they were willing to cooperate. All cooperating farms were then visited individually by the same interviewer, starting in October 2009. This paper shows the data gathered until January 2010. The questionnaire contained a variety of questions, related to gilt management, batch management system, weaning practices, heat stimulation and detection, insemination, farrowing, housing, climate, feeding, hygiene, vaccinations and fertility parameters. Each visit lasted approximately one hour.

Results

Table 1 shows the descriptive results of important parameters of 30 herds located in three provinces in Flanders. These provinces represent 94% of the Belgian pig production. Of the 30 herds, 37% were farrow-to-finish farms. An equal number of farms purchased gilts or selected their own gilts. Farm size distribution was as follows: 10% less than 150 sows, 63% between 150 and 300 sows and 27% more than 300 sows. The majority (57%) of the farms used a batch management system, mainly 3 and 4 week systems.

Table 2 shows the mean, standard deviation and range (minimum, maximum) of the major fertility parameters.

Discussion and conclusion

As these are preliminary results on a limited number of farms, correlations are not yet clearly visible. By mid 2010, the study will be finalized and the final results and a risk factor analysis will be presented.

Parameters		%
Weaning	<21 days	7
	21-24 days	43
	25-28 days	50
Number of teaser boars/ 20 sows	<1	23
	1	40
	2	23
	>2	13
Heat stimulation starting from	The day of weaning	23
	day 1 after weaning	50
	>day 1 after weaning	27
Heat stimulation	<2x/day	43
	2x/day	53
	>2x/day	3
First heat detection	<day 4 after weaning	13
	day 4 after weaning	70
	>day 4 after weaning	17
Heat detection	<2x/day	17
	2x/day	83
Light at sow-height in insemination unit >150lux	yes	50
Feed weaned sows	Pregnancy feed	57
	Lactation feed	33
	Other	10

Table 1: Descriptive results of 30 herds

	mean	st dev	min	max
Weaned piglets/sow/year	25.82	2.78	21.32	31.44
Weaning-to-oestrus interval	6.18	1.19	4.64	9.27
Litters/ 100 inseminations	82.6	4.39	75.1	90.8
Pregnancy rate after 1st insemination	91.78	5.95	71.9	97.6

Table 2: Major reproduction parameters of 30 herds

Acknowledgements

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EFFECT OF TEMPERATURE AND PACKING METHOD ON STORAGE OF BOAR SEMEN

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Introduction and objectives

Storage of semen has an irreversible effect on its quality (1). Boar spermatozoa are very sensitive to "cold shock" (2). If semen temperature suddenly drops below 15°C, most spermatozoa do not survive (3, 4). After dilution, semen is cooled until 17°C. At this temperature, it can be stored without causing damage (5). The purpose of this study was to investigate whether the storage of boar semen at different temperatures and in different packages influence spermatozoa quality.

Materials and Methods

The experiment was performed in April 2009. Ejaculates (dilution with Minitub BTS® until $2.5x10^9$ at 26°C) of 6 Piétrain boars from a commercial AI centre (1 ejaculate/boar), were packed in 2 dissimilar assemblages: blisters (most frequently used: 85% market share) and Gédis® (Genes Diffusion: 12% market share). The semen samples were transported to the laboratory of the Faculty of Veterinary Medicine in Merelbeke, kept at room temperature for two more hours and then stored at 3 distinct temperatures: 14°C, 17°C (standard) and 20°C. To determine the quality of the spermatozoa, the following aspects were investigated: motility, progressive motility, percentage normal and percentage live spermatozoa. The first two parameters were analysed using the CASA (Computer Assisted Semen Analyzer) daily from D0 (semen collection) to D4. The percentage of normal and live spermatozoa was also examined daily in a blinded way using eosin-nigrosin staining. Statistical analysis was performed by means of a linear mixed effects model, in which boar was the random effect (S+).

Results

Table I shows the outcomes (mean over the five days) of the four investigated parameters of the semen, stored at different temperatures in blisters. Table II shows the same, but with semen packaged in Gédis®.

Over the five days, a significant lower percentage live and normal spermatozoa was seen in semen packed in Gédis® in comparison to that in blisters at 17°C ($p < 0.05$).

In case of extended preservation of the semen (> 2 days), a significant decrease in the percentage live (90.3% vs. 86.1%) and normal spermatozoa (84.7% vs. 80.4%), motility (67.1% vs. 57.4%) and progressive motility (45.9% vs. 32.5%) was observed in both packing methods and at all temperatures ($p < 0.01$).

Table I: Mean outcomes of semen stored in blisters

	14°C (ref.)	17°C	20°C
% live spermatozoa	89.4	91.5*	91
% motile spermatozoa	63.5	60.3	57.9*
% progressive motile sp.	36.1	37.3	37.3
% normal spermatozoa	83.3	85.7	86.1

*: significantly different from reference category within row ($p < 0.05$)

Table II: Mean outcomes of semen stored in Gédis

	14°C (ref.)	17°C	20°C
% live spermatozoa	83.7	87.1*	84.1
% motile spermatozoa	64.6	61.9	59.4*
% progressive motile sp.	40.4	37.4	38.6
% normal spermatozoa	78	81.5	78.6

*: significantly different from reference category within row ($p < 0.05$)

Discussion and conclusion

These results show that the quality of boar semen decreases when stored for a longer period than two days as well as when it is preserved at temperatures lower or higher than 17°C. The present study demonstrates also a better longevity when spermatozoa are packed in blisters than in Gédis. To investigate the fertility results of semen stored in these conditions, more *in vivo* studies are needed.

Acknowledgements

The authors are grateful to AI centre Hypor to provide us with boar semen.

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ASSESSMENT OF BIO-SECURITY MEASURES ON A PIG FARM

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Introduction

Intensive pig production means housing a large number of animals of various ages in a buildings wth a limited space per animal, which has both advantages in terms of the efficiency. Of the production (lower costs per kg produced pork) and higher risks in terms of disease prevention and control. The husbandry conditions of many animals of various ages under one roof, combined with the presence and circulation of a large number of organisms causing disease (such as bacteria, viruses and parasites), adverse microclimate conditions in the facilities (which compromise the immune system), non-compliance with hygiene and sanitary measures, qualitative and quantitative deficiencies in the supply of feed and drinking water, often leads to infectious as well as noninfectious diseases and reproductive disorders that not seldom are due to infections such as PRRS and others.

Material & Methods

The aim of this study was to identify the most sensitive places concerning biosecurity on a modern pig farm with 250 sows in reproduction, breeding sows and breeding boars. - After assessing the measures already in place and of the missing biosecurity measures the results were presented to the owner in order to discuss possibilities of improventment. The regulation on veterinary sanitation of facilities for breeding ungulates, poultry and rabbits, which is part of the Veterinary Law (Official Gazette of RS, no. 91/05) was the basis of the assessment and the criteria of this regulation were used during the assessment.

Results

Several sites of breaches in biosecurity were found which might endanger the health of the pigs on this particular farm. These sensitive sites were:

- the inadequate size and layout of the disinfection barriers for cars and personnel (no-coverage and with length less than 6 m, and a depth less than 25 cm),
- no possibility for the workers to use clearly defined and clean passage ways,
- the lack of a loading ramp which allows customers to have direct contact with the workers during the sale of sows and boars,
- the lack of safety nets to the fan openings,
- the lack of three lines of defense against rodents,
- the lack of employing liquid and solid rodenticides outside the barn and along the fence.,

Discussion & Conclusions

Multiple deficiencies in the primary and secondary biosecurity measures were found at this particular pig farm. Suggestions to solve these problems were made in a report to the owner of the farm. Since every measure that was suggested was accompanied by an explanation about why each specific measure was important to maintain the health of the animals, the results of this analysis can be generalised as instructio for the improvement of pig afrms that have grown to a bigger size without having implemented appropriate biosecurity measures.

SERUM MICRO-ELEMENT REFERENCE VALUES IN SOWS AT DIFFERENT STAGES DURING THE REPRODUCTIVE CYCLE

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Introduction and Objective

The measurement of serum macro-element parameters of farm animals can provide important information on health and metabolism of several organ systems (1) and is a practical diagnostic tool for assessing pathological conditions in the live animal or for monitoring the health status of groups of animals (2). Recently, an episode of reproductive problems, without specific infectious cause, was observed in several pig herds in Flanders. No major shortcomings were observed in management and housing conditions. Besides infectious causes of the reproductive problems, feeding strategies and feed composition could have an impact on reproductive performance of the sows. Several components such as vitamin E, selenium (Se) and minerals (Ca, Mg, Mn, Cu and Zn) could have an influence on reproduction (1). However, to interpret the obtained results in serum macro- and micro-element analysis, reliable reference values should be available. Therefore, blood was collected from primiparous and multiparous sows in several farms with no reproductive problems in order to establish reference values for several minerals and vitamin E. The effect of stage of reproductive cycle was also evaluated.

Materials and Methods

Seven pig farms with at least 100 sows in a batch management system (BMS) (3-, 4- or 5-week BMS) were selected based on the absence of reproductive problems. The herds had between 200-450 sows. Samples were collected from the same sows at 60 ± 5 d of gestation, 3 ± 1 d after farrowing and at 3 ± 1 d after weaning. All samples were stored at -20°C until the end of the sampling period and analysed subsequently in one single batch. Analysis was performed as follows: Se (µg/l) and Mn (µg/l) using an atomic absorption technique and vitamin E (mg/dl) using HPLC-analysis.

Results

Results expressed as mean and range (2.5-percentile and 97.5-percentile) for primiparous and multiparous sows (Table 1 & 2). Results for 60 d of gestation were in between 3 d in lactation and 3 d post-weaning and are not shown here.

Table 1. Reference values for serum macro-elements of the parity-one sows (n = 14) and parity ≥ 2 sows (n = 31) from 7 commercial pig herds at 3 ± 1 d of lactation

	Parity-one sows		Parity ≥ 2 sows	
	Mean	**Range**	**Mean**	**Range**
Mn	1.07	0.45-2.14	1.24	0.34-2.70
Se	174.64	125.75-239.50	166.42	125.75-237.75
VitE	2.01	1.34-2.65	1.93	0.80-2.93

Table 2. Reference values for serum macro-elements of the parity-one sows (n = 14) and parity ≥ 2 sows (n = 31) from 7 commercial pig herds at 3 ± 1 d after weaning

	Parity-one sows		Parity ≥ 2 sows	
	Mean	**Range**	**Mean**	**Range**
Mn	2.02	0.64-4.70	2.30	0.61-6.13
Se	188.73	141.75-237.50	177.84	135.75-234.25
VitE	3.61	1.88-5.12	3.44	1.72-5.32

Discussion and Conclusions

It is difficult to compare the obtained results with previous results due to differences in analytical techniques (3). The reference values obtained for vitamin E were lowest at parturition and increased at weaning, whereas values of vitamin E at 60 d of gestation were in between the previous ones. Selenium reference values had a quite stable kinetics with slightly higher values at 3 d after weaning. For Mn, the lowest reference values were observed at 3 d of lactation. Overall, primiparous sows had lower reference values as compared to multiparous sows in all three production stages.

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SERUM MACRO-ELEMENT REFERENCE VALUES IN SOWS AT DIFFERENT STAGES DURING THE REPRODUCTIVE CYCLE

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Introduction and Objective

The measurement of serum macro-element parameters of farm animals can provide important information on health and metabolism of several organ systems (1) and is a practical diagnostic tool for assessing pathological conditions in the live animal or for monitoring the health status of groups of animals (2). Recently, an episode of reproductive problems, without specific infectious causes, was observed in several pig herds in Flanders. Several minerals could have an influence on reproduction (1). However, to interpret the obtained results in serum macro-element analysis, reliable reference values should be available. Therefore, blood was collected from primiparous and multiparous sows in several farms with no reproductive problems in order to establish reference values for several minerals. The effect of stage of reproductive cycle was also evaluated.

Materials and Methods

Seven sow farms with at least 100 sows in a batch management system were selected based on the absence of reproductive problems. Samples were collected from the sows at 60 ± 5 d of gestation, 3 ± 1 d after farrowing and at 3 ± 1 d after weaning. Analysis was performed as follows: Na, Cl, K (mmol/l) using Vetlyte (Idexx), P (mmol/l) using Vettest (Idexx), Fe (µmol/l) using Diasys nl Iron FS Ferene (Konelab TX20), Cu (µmol/l) using Randox (Konelab TX20) and Ca and Mg (mmol/l) using Siemens Medical Solutions (Konelab TX20) and Zn (µg/dl) using an atomic absorption technique.

Results

Results expressed as mean and range (2.5-percentile and 97.5-percentile) are given for primiparous and multiparous sows in Table 1-2. The results of 3 d after weaning were comparable to those at 60 d of gestation and were therefore not presented here.

Discussion and Conclusions

It is difficult to compare the obtained results with previous results due to differences in analytical techniques (3).

Table 1. Reference values for serum macro-elements of the parity-one sows (n = 14) and parity ≥ 2 sows (n = 31) from 7 commercial pig herds at 3 ± 1 d of lactation

	Parity-one sows		Parity ≥ 2 sows	
	Mean	**Range**	**Mean**	**Range**
Ca	2.58	1.97-2.88	2.63	1.65-3.07
P	2.13	1.44-2.62	2.03	1.23-2.62
Mg	1.13	0.75-1.52	0.99	0.39-1.26
Na	138.3	101.5-151.0	139.2	84.2-154.3
Cl	99.45	76.75-105.50	99.13	64.50-110.25
K	7.85	5.12-12.23	6.75	3.60-11.52
Cu	37.36	25.92-48.02	36.49	21.67-47.13
Zn	78.64	49.25-111.00	71.16	44.75-116.25
Fe	41.39	14.02-88.5	32.67	14.82-60.75

Table 2. Reference values for serum macro-elements of the parity-one sows (n = 14) and parity ≥ 2 sows (n = 31) from 7 commercial pig herds at 60 ± 5 d of gestation

	Parity-one sows		Parity ≥ 2 sows	
	Mean	**Range**	**Mean**	**Range**
Ca	2.67	2.32-3.01	2.74	2.44-2.98
P	2.68	2.14-3.44	2.38	1.77-3.70
Mg	1.19	1.06-1.32	1.14	0.98-1.39
Na	145.3	137.5-152.3	144.5	133.2-150.3
Cl	104.0	101.0-107.0	104.2	97.25-108.25
K	9.00	6.70-12.77	8.17	5.07-13.25
Cu	37.28	28.00-43.70	34.39	27.52-41.65
Zn	84.64	66.75-118.25	77.26	52.50-100.75
Fe	42.55	32.47-70.88	40.10	21.15-75.08

The reference values obtained for most of the determined elements were slightly higher at 60 d of gestation as compared to 3 d in lactation. Moreover, the ranges are narrower at 60 d in gestation, indicating less variation within the animal population. Overall, primiparous sows had higher reference values as compared to multiparous sows in lactation and during gestation.

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EFFECTS OF IRON AND COPPER SUPPLEMENTATION DURING ADVANCED PREGNANCY OF SOWS ON THE HAEMATOLOGY OF PIGLETS

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Introduction: Piglets in modern husbandry practices are born with very low iron reserves and become anaemic if additional iron is not provided parenterally or orally. The young pig uses its own iron reserves along with the iron supplied through mother's milk to meet its iron need but these supplies are normally inadequate. Efforts have been made to raise the level of iron in piglets through different means and the most successful results have been obtained with intramuscular injection of iron-dextran in newly born piglets. However, this practice is very stressful and the piglets suffer from pain (Roberts 1998). Keeping in mind the role of iron and copper in haemoglobin synthesis, this study was carried out to evaluate the effect of supplementation of these elements in pregnant sows on the haematology of their offsprings.

Materials and Methods: Three Large White Yorkshire sows (first to third parity) were selected randomly and kept individually with litters in farrowing pens having concrete flooring. They were managed on similar pattern except iron and copper supplementation. The control sow (T_0) was supplemented neither for iron nor for copper. One injection of iron-dextran (1500 mg elemental iron) was administered on 110th day of gestation in T_1 sow, while three injections of iron dextran (500 mg elemental iron each) on 106th, 109th, and 112th day of gestation to the T_2 sow. In addition, T_2 sow was supplemented with 2.4 g copper sulphate, pentahydrate through concentrate feed daily for 7 days prior to farrowing. Blood samples were collected on 5th and 8th day of age for estimation of haemoglobin and total erythrocyte count (TEC) from five piglets selected randomly from each sow. All the piglets were injected intramuscularly with 100 mg iron as iron-dextran on 9th day of age.

Results: Litter size was 8, 7 and 11 in T_0, T_1 and T_2, respectively. Haemoglobin concentration was higher (p 0.01) in T_2 piglets in comparison to the piglets of other treatments. The average value of haemoglobin was 9.0 ±0.30, 9.56± 0.34 and 10.16 ±0.26 g/dl on 5th day of age and 8.16±0.38, 9.52±0.33 and 10.72±0.34 g/dl on 8th day of age in T_0, T_1 and T_2 piglets, respectively. The haemoglobin level in T_1 and T_2 was normal but it progressively decreased (p 0.05) in T_0 up to 8th day of age. TEC was also higher (p 0.01) in T_2 piglets on same age basis. It was 3.63 ±0.40X10^6, 3.19± 0.24X10^6 and 4.70 ±0.41X10^6 per cu mm of blood on 5th day and 2.93±0.20X 10^6, 3.42±0.19X10^6 and 4.74±0.30X10^6 per cu mm of blood on 8th day of age in T_0, T_1 and T_2 piglets, respectively. The trend in change of haemoglobin and TEC was similar up to 8th day of age.

Discussion and Conclusions: Iron is an integral constituent of haemoglobin while copper is essential for its formation and iron transport (Nazifi et al. 2005). The decrease in haemoglobin concentration during first week of life in T_0 piglets was in accordance with other findings and was attributed to the increase in circulatory fluid and dilution of blood haemoglobin concentration in growing piglets (Peters and Mahan 2008). In spite of larger litter size in T_2, the higher haemoglobin and TEC values indicated that the Cu supplementation is essential along with the Fe for maintaining their normal physiological levels and longer survival rate of RBCs. It was concluded that supplementation of iron and copper during last week of gestation is beneficial for maintaining normal haemoglobin levels and TEC in new born piglets. This may help in delaying first injection of iron-dextran till 9th day of age to reduce stress on new born piglets.

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EFFECT OF AMINO ACID RESTRICTION IN DIFFERENT FEEDING PHASES ON CARCASS QUALITY OF GROWING-FINISHING GILTS

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Introduction

Optimizing the feeding strategy by closely matching supply to requirement seems most efficient, both economically and environmentally. However, another strategy could be to limit dietary protein or energy as this can evoke a compensatory growth response. Therefore, the present experiment aimed at investigating the effect of dietary amino acid restriction between 20 and 70 kg or between 70 and 110 kg on carcass quality in a lean meat type of growing-finishing gilts.

Material & Methods

Ninety-six gilts (Piétrain boar x hybrid sow) were selected and divided over sixteen pens. Each pen was randomly assigned to one out of four dietary treatments: 1) high amino acid (AA) levels in growing (20-40kg) and early finishing (40-70 kg), with high AA level in late finishing (70-110 kg) (HH); 2) high AA levels in growing and early finishing, with low AA level in late finishing (HL); 3) low AA levels in growing and early finishing, with high AA level in late finishing (LH); and 4) low AA levels in growing and early finishing, with low AA level in late finishing (LL). Diets were isoenergetic and pigs were fed *ad libitum*. The high AA diet was formulated to yield good performances (Millet et al., 2010). The low amino acid diet contained 20% (growing diet) or 30% (finishing diets) less lysine, threonine, methionine and tryptophan. At the slaughterhouse, optic light measurements were performed with a 'Capteur Gras-Maigre' device (CGM) equipped with an 8 mm diameter Sydel probe (SYDEL, Lorient, France). Lean meat content in the carcass was estimated based on this CGM measurement with the equation approved for use in Belgian slaughterhouses. Carcass yield was calculated as cold carcass weight divided by live weight before transport to the slaughterhouse.

Results

Restricting the AA level in the late finishing phase decreased carcass yield and muscle thickness of the carcasses (both $P<0.01$), consequently leading to a lower meat percentage ($P<0.01$) (Table 1). A tendency to a higher fat thickness was seen ($P=0.06$). A tendency to an interaction between amino acid content from 20-70 kg and from 70-110 kg was noticed for meat percentage ($P=0.08$) and muscle thickness ($P=0.07$), with a larger difference between the LH and LL group than between the HH and HL group.

Table 1. Carcass quality for four treatment groups

Treatment group	HH	HL	LH	LL	SEM
Carcass yield (%)	79.3	78.6	79.2	78.2	0.1
Muscle thickness (mm)	69.5	67.3	70.9	64.6	0.6
Fat thickness (mm)	11.4	11.5	11.3	12.2	0.3
Meat percentage (%)	63.7	63.1	64.2	61.7	0.3

Discussion & Conclusion

Even though we used a lean meat type of pig and a longer period of restriction than in other studies (Reynolds et al., 2006; Fabian et al., 2002), amino acid restriction in the growing and early finishing phase did not affect carcass quality, which agrees with these studies. It can be expected that AA restriction in the growing and early finishing phase limits lean tissue deposition. However, the present results suggest that the pigs can compensate for this if the AA level in the late finishing phase is sufficient. The lower carcass yield in the group with AA restriction in the late finishing phase may be explained by an increased deposition of abdominal fat at the expense of muscle tissue.

As a conclusion, a balanced diet during the late finishing phase is crucial to obtain good carcass quality of pigs.

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CARCASS TRAITS AND BEHAVIOUR OF SURGICALLY CASTRATED AND IMPROVAC® VACCINATED BOARS

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Introduction

Active immunization against gonadotropin-releasing hormone (GnRH) is an alternative to surgical castration especially from a welfare point of view. The objective of the present study was to compare behaviour and carcass traits of male fattening pigs either surgically castrated without anaesthesia or vaccinated twice with Improvac® (GnRH-analogue vaccine against boar taint) under commercial German fattening conditions.

Material & Methods

Boars were either surgically castrated without anaesthesia within the first week of life or vaccinated against GnRH twice at 10 and 21 weeks of age (1). Each treatment comprised 8 groups of 12 pigs, housed in fattening pens (2, 3).

Data on postures of the animals were scored from 24-hour videos recorded in every week of the fattening period (16 weeks) using scan sampling with 5 minute intervals. Additionally social behaviour was analysed in weeks 2, 4, 6, 8, 10, 12, 14, 15 and 16 by continuous behaviour recording of focus animals in four blocks.

Animals were slaughtered at 25 and 26 weeks of age respectivley. Carcass traits (kg carcass weight, % lean muscle, mm loin muscle and backfat thickness) were recorded, and intensity of boar taint was assessed organoleptically by two independent institutions.

Results

During the whole fattening period, vaccinates were more active than surgical castrates. Vaccinated animals showed a significant decrease in standing ($P<0,023$) and an increase of sitting and lying after the second vaccination of Improvac. No significant effects of treatment on the total number of agonistic interactions and on biting and fighting were found. In vaccinates the prevalence of aggressive behaviour parameters decreased significantly after the second vaccination ($P<0,001$).

Organoleptic anomalies, particularly pronounced sexual odour, as assessed in a blinded examination by two veterinary authorities, were not detected in any of the carcasses.

Vaccinated animals markedly differed from surgically castrated animals in carcass weight, [97,67 ±0,90 kg / 94,81 ±0,89 kg]. Carcasses of Improvac treated boars were significantly leaner [lean meat (%): 56,41 ±0,36 / 54,06 ±0,38; loin muscle (mm): 58,70 ±0,64 / 56,93 ±0,67 and had less backfat (mm): 15,77 ±0,42 / 18,25 ±0,44] than those of surgical castrates.

Discussion & Conclusions

Our results demonstrate that under commercial German pig fattening management boar taint can be prevented and carcass quality can be improved by the use of Improvac. Housing of male pigs vaccinated against GnRH in single sex groups of 12 individuals does not increase behavioural problems in the fattening period compared with surgically castrated males.

GnRH vaccination of male fattening pigs is an animal welfare-friendly alternative to surgical castration because it avoids surgical procedure, which is associated with pain and stress even when performed under local or general anaesthesia.

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