

Proceedings 1st ESPHM

27-28 August 2009

FACULTY OF LIFE SCIENCES
COPENHAGEN · DENMARK



ECPHM

EUROPEAN COLLEGE
OF PORCINE HEALTH
MANAGEMENT

FACULTY OF LIFE SCIENCES
UNIVERSITY OF COPENHAGEN

PROCEEDINGS

The 1st European Symposium on Porcine Health Management

27- 28 August 2009

Copenhagen, Denmark

Edited by

Helle Stege, Faculty of Life Sciences, University of Copenhagen

Charlotte Sonne Kristensen, Danish Agriculture and Food Council, Danish Pig Production

Proceedings of the 1st ESHPM

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PREFACE

It is a great pleasure to welcome you to the 1st European Symposium on Porcine Health Management (ESPHM) at the Faculty of Life Sciences, University of Copenhagen in 2009. We are very pleased that more than 200 delegates from 16 European countries, the United States, Latin America, Australia and the Middle East have registered for the symposium, by mid August. Apart from 25 invited key note lectures and speakers we have received 40 abstracts to be presented as posters. It is our aim that participant interaction, fruitful discussions and social networking will dominate the symposium. As a novelty, designated persons have been asked to function as so-called opponents after each key-note lecture in order to stimulate discussion and reflection.

We hope the 1st ESPHM will be the first of a long line of similar meetings, and thereby create an important way of exchanging knowledge and stimulating cooperation within porcine health management at the international level. There are at least three reasons for the present ESPHM duckling, ugly or not, to grow into a long living swan.

First, the European pig production is of considerable size and economic importance. In 2008, the pig inventory in the 27 EU countries was 160 million head. The number of pigs slaughtered was 254 million each year. This makes the EU pig production the largest in the western world followed by the 82 million head inventory and 137 million slaughtered pigs in North America (United States and Canada). The corresponding 440 million head inventory in China is then another story. Evidence based health management, as presented at the ESPHM, is a prerequisite for survival of pig production in the global and regional competition.

Second, there is an ongoing development in the pig industry, supported by the societies and legislators, towards production in environmental friendly production units with loose housing systems, prudent use of antibiotics, gentle animal handling procedures and care for diseased animals. Health, welfare, food safety including antibiotic resistance in such units is closely linked to the occurrence of infectious and non-infectious diseases. This creates challenges to those involved in health management and furthermore increases the need for the results presented at the ESPHM.

Third of all, the infectious disease and zoonosis situation in countries outside and within the pig producing regions such as the EU must be considered continuously. The emergence of old and new diseases due to changes in disease epidemiology or climatic conditions, calls for a regional approach. New information on these topics will be presented at the ESPHM.

The well established European College of Porcine Health Management (ECPHM) will help to anchor, organize and support the ESPHM. The ECPHM was founded in 2004 and has now approved more than 100 European Veterinary Specialists in porcine health management (diplomates). Furthermore, 9 ECPHM residency programmes have been established at European universities and ECPHM exams have been carried out twice.

We hope that all participants of the 1st ESPHM will enjoy a scientifically stimulating and socially appealing symposium.

Jens Peter Nielsen,
President 1st ESPHM

ACKNOWLEDGEMENTS

A symposium as the 1st ESPHM 2009 would not have been possible without the financial and professional support of our Congress Partners and Sponsors.

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We 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 invited keynote lectures and invited lectures on selected important topics. Exchange of new information is the core business of the symposium.

The International Scientific and Organizing Committee, the European College of Porcine Health Management, the Faculty of Life Sciences-University of Copenhagen, the Danish Agriculture and Food Council-Danish Pig Production and the Danish Veterinary Association is thanked for assistance and support throughout the planning and organizing process.

Finally a special thank goes to Secretary Jeanne Talchow Oakman from the Faculty of Life Sciences for her great efforts and professionalism in preparing the proceedings and many other practical issues in relation to the symposium.

INTERNATIONAL SCIENTIFIC AND ORGANIZING COMMITTEE

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Programme - 1st European Symposium on Porcine Health Management - Copenhagen 2009

Aug. 27 th	Session	Type	Title of presentation	Min.	Speaker/opponent
8.30- 9.15	Registration				
9.15- 9.30	Welcome and opening by the dean of the Faculty of Life Sciences			15	<i>PH Andersen</i> <i>JP Nielsen</i>
9.30-10.15	Porcine health management in practice <i>Chairman Jens Peter Nielsen</i>	KNL*	The role of the swine practitioner in the future	25	<i>K Truschner</i>
		IS**	Opponent –swine practice	10	<i>C Heisel</i>
			Plenary discussion	10	
10.15-10.45	Poster viewing (coffee, water, fruit)				
10.45-12.30	Infectious reproductive problems in the sow <i>Chairman Dominiek Maes</i>	KNL	PCV2, PRRSV, influenza, PPV and other viruses	40	<i>H Nauwynck</i>
		IS	Opponent - viral infections	10	<i>P Martelli</i>
			Plenary discussion	10	
		KNL	<i>Leptospira, Erysipelotrix, E. coli and A. suis</i>	25	<i>GP Martineau</i>
		IS	Opponent – bacterial infections	10	<i>SE Jorsal</i>
	Plenary discussion	10			
12.30-14.00	Lunch				
14.00-15.15	Non-infectious reproductive problems in the sow <i>Chairman Olli Peltoniemi</i>	KNL	Management and nutrition	40	<i>D Maes</i>
		IS	Opponent – management	10	<i>F Thorup,</i>
		IS	Opponent – nutrition	10	<i>C Oliviero</i>
			Plenary discussion	15	
15.15-15.45	Poster viewing (coffee, water, fruit)				
15.45-16.45	ECPHM residents presentations <i>Chairman Arlette Laval</i>	IS	Porcine ulcerative dermatitis syndrome (PUDS)	15	<i>A Lopez</i>
		IS	<i>H. parasuis, M. hyorhinis</i> and polyserositis in pigs	15	<i>A Palzer</i>
		IS	Sow mortality in Belgian farms: Diagnostic findings	15	<i>F Vangroenweghe</i>
		IS	<i>L. intracellularis</i> and <i>Brachyspira</i> in Swiss pig farms	15	<i>F Zeeh</i>
19.00-23.00	Symposium Dinner Garden of Frederiksberg				

Aug. 28 th	Session	Type	Title	Min.	Speaker
9.00-10.15	Emerging diseases <i>Chairman Joaquim Segales</i>	IS	African Swine Fever outbreaks close to EU	15	<i>A Utenthal</i>
		IS	New neonatal diarrhoea syndrome France	15	<i>GP Martineau</i>
		IS	New neonatal diarrhoea syndrome Denmark	15	<i>B Svensmark</i>
		IS	Gastric ulcers in pigs: Do bacteria play a role?	15	<i>F Haesebrouck</i>
			Plenary discussion	15	
10.15-10.45	Poster viewing (coffee, water, fruit)				
10.45-12.00	Emerging zoonosis <i>Chairman Thomas Blaha</i>	IS	Influenza A in pigs	15	<i>LE Larsen</i>
		IS	MRSA in pigs and in occupationally exposed persons	15	<i>D Meemken</i>
		IS	Risk analysis of porcine zoonosis	15	<i>L Alban</i>
		IS	Hepatitis E in pigs	15	<i>J Segales</i>
			Plenary discussion	15	
12.00-13.00	Lunch				
13.00-15.00	Diarrhoea in growers and finishers <i>Chairman Poul Bækbo</i>	KNL	Pathology of enteritis in pigs	40	<i>TK Jensen</i>
		IS	Opponent – enteritis	10	<i>D Taylor</i>
		IS	<i>Brachyspira</i> infection in pigs	30	<i>C Fellström</i>
		IS	Diagnosis of enteritis in growers	20	<i>K Pedersen</i>
			Plenary discussion	20	
15.00-15.15	Conclusion of meeting			15	<i>JP Nielsen & T Blaha</i>

- * KNL= Key Note Lecture

- ** IS = Invited Speaker

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THE ROLE OF THE SWINE PRACTITIONER IN THE FUTURE

K Truschner

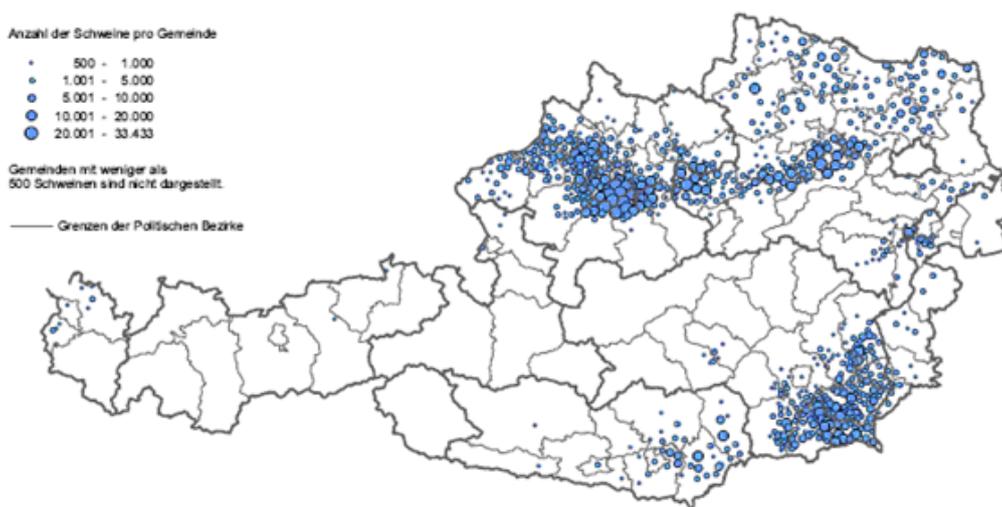
Diplomate of ECPHM

The role of a pig veterinarian has changed completely over the last 30 to 40 years. 40 years ago veterinary work was like the work of a “fire brigade”. One individual animal or a whole herd (at that time mostly small herd sizes) had a health problem and the veterinarian tried to treat just the disease without taking other things like management, ventilation, food, biosecurity, etc. into consideration. Due to this situation the veterinarian had to be available twenty-four-seven throughout the year. It was also necessary to have the veterinary practice close to the farm, because of frequent acute problems (birth, red murrain, traumas,...).

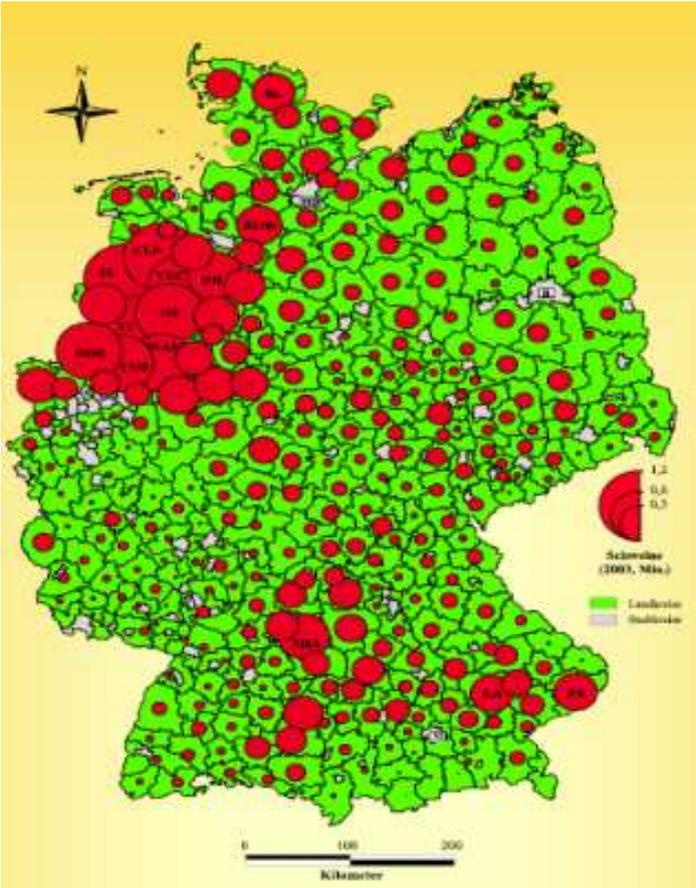
In the present there are a lot of changes in Austrian swine production. Since Austria joined the EU in 1995 60 % of our pig producers went out of production. Even we have the worst structure of herd sizes (on average 28 sows / farm) in the European Union the “survivors” are trying to increase.

At the moment there are 3 main regions in Austria with 36 700 producers. 91% of the Austrian swine herds are located in Upper Austria, Lower Austria and Styria.

Concentration of pig production in Austria



Concentration of pig production in Germany



Industrial farms



pictures: Noe

Those dramatic changes in structure (as we experienced it in the past only due to epizootics) require permanent diagnostic investigations and therefore also changes in our veterinary services. We have to meet the new requirements of our clients. As in other countries beside the “fire brigade” work, pig veterinarians must have also detailed knowledge in herd health management, biosecurity, planning of pig barns and agricultural indoor facilities and understanding the ventilation and feeding systems.

Very important is not only an efficient cooperation between the veterinarian and the farmer, but also with laboratories and scientific institutions (universities).

The work of the pig veterinarian in the future has to be adopted on the structure of a global swine industry. Small farms are going out of production and are replaced by larger farrow-to-finish units (Eastern Europe, Russia, Asia). Although in Europe the family farm production model is still predominant, large companies whose production is based on the integration model are becoming more (Spain). Because of larger distances between those farms the veterinarians have to show more flexibility in travelling around the world and working in different places. One of the future key points will be to take a leading position in herd health management, animal welfare, public health and environment.

Veterinarians also have to develop new strategies of problem solving with new technologies of communication like Internet and video meetings where distances to the farms are not an issue.

All these new requirements demand a changed way of education on the universities (herd health management, economic cost calculation, European legislation in animal production, pharmaceutical and immunological knowledge on herd basis ...) and therefore also new education and training programs for the farmers, managers and stock people of pig producing companies.

VIRAL REPRODUCTIVE PROBLEMS IN THE SOW

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Gestation can only be sustained by (i) a correct hormonal balance (high progesterone concentration), (ii) local cytokine and growth factor crosstalk between uterine leukocytes (especially cells of the macrophage lineage and NK cells), endometrial cells and embryonic/fetal macrophages and chorionic cells, and (iii) a strong suppression of the immunity at the level of the implantation region of the uterus (Arck et al., 2007; Wessels et al., 2007). At the end of gestation, when the fetus is mature enough to be born, it starts to produce corticosteroids that in turn induce prostaglandins in the placenta (Silver and Fowden, 1989; Burchard et al., 1992). These prostaglandins break down the corpora lutea and as a result the concentration of progesterone drops. As a consequence, the fibrous tissues of the reproductive tract soften and the motility of the uterus becomes activated. It is generally believed that the immune suppression is no longer supported, resulting in a response against the paternally-inherited antigens on tissues of the fetus and that the inflammatory reaction allows a slow release of the fetal placental membranes from the endometrium. All these changes end up with the birth of the piglet. Every deviation on this normal situation may lead to interruption of gestation and death of the embryo/fetus (Tayade et al., 2007, Wessels et al., 2007).

Viral infections during gestation in sows are frequently causing reproductive failure, characterized by embryonic and fetal death, return to oestrus, abortion, early or late farrowing and birth of mummies, weak- and stillborn piglets. How viruses induce these reproductive problems is complex and quite different in between viruses. Since there is still a lot of information lacking on the normal physiology/immunology of gestation, it is not always easy to find sound explanations for the reproductive problems. In the present overview, the pathogenesis of reproductive failure during viral infections will be reviewed.

Viruses may affect normal gestation in two ways. They may cause reproductive problems in an indirect way when they replicate in distant areas and affect gestation via host factors or in a direct way by replicating in the genital tract and/or embryonic/fetal tissues.

Indirect reproductive failure - A typical virus that gives indirect reproductive problems is swine influenza virus (SIV). This virus replicates to high titers in the upper respiratory tract and causes an acute cytokine burst (Van Reeth et al., 1998). Especially the pro-inflammatory cytokines interferon-alpha, tumor necrosis factor-alpha and interleukin 1 peak at high levels, leading to severe general clinical signs such as inactivity, anorexia and high fever. During this stage, abortion may be seen. The aborted fetuses are fresh and mostly in a state of rigor mortis (firm). It is generally accepted that the high concentration of pro-inflammatory cytokines causes a fast breakdown of the fragile tolerance balance in between mother and embryo/fetus.

Direct reproductive failure - Several viruses are able to replicate in the reproductive tract and/or embryos/fetuses. The most important RNA viruses are: porcine reproductive and respiratory syndrome virus (PRRSV), classical swine fever virus (CSFV), porcine enteroviruses (PEV) and porcine encephalomyocarditis virus (EMCV); the most important DNA viruses are: porcine parvovirus (PPV), porcine circovirus type 2 (PCV2) and Aujeszky's disease virus (ADV).

These viruses may reach the embryos/fetuses via two ways: (i) via contaminated semen during natural service or artificial insemination and/or (ii) via blood. The viruses that are shed via semen are: PRRSV, CSFV, PPV, PCV2 and ADV (Maes et al., 2008). Semen contaminated with these viruses may lead to infection of embryos, causing embryonic death and return to estrus of the sow, except for PRRSV. Mateusen et al. (2007) showed that the refractory state of embryos to PRRSV infection can be explained by the absence of its receptor sialoadhesin. All viruses that are mentioned cause a viremia and may cross the placenta (Pensaert et al., 2004). Due to the presence of a firm placenta barrier that does not allow large proteins, such as antibodies, to cross, it is difficult to believe that bigger structures, such as virus particles may do this. Therefore, two main ways to cross the placenta are proposed: (i) via infected blood leukocytes that upon adhesion to uterine endothelial cells migrate through the placental layers or allow viral spread from cell to cell up to the fetal tissues (ADV, CSFV, PCV2) or (ii) via blood leukocytes that get infected by cell-free virus at the moment that they adhere to the endothelial cells of the

placenta and subsequently migrate through the placental layers (PRRSV, PPV, PEV, EMCV). The clinical outcome of the transplacental crossing of the virus for the fetus and the sow differs largely on the type and titer of the virus, the stage of infection and the number of fetuses that are simultaneously infected.

Transplacental spread of ADV and virulent CSFV strains leads to a quick spread of the virus in the endometrium, the fetal placenta and the different organs of fetuses (Nauwynck et al., 1992; Dewulf et al., 2001). This frequently leads to abortion during the whole gestation period, even after 70 days. The fetuses are fresh or partially mummified (brown discoloration). Further stages of mummification (black discoloration) may occur. Low virulent CSFV strains may lead to immunotolerance in fetuses when the infection occurs before 70 days of gestation and teratogenic effects.

During the transplacental spread, PCV2, PPV, PEV and EMCV are not replicating to a high extent in the endometrial tissues and are not damaging the placenta. Furthermore, the viruses are spreading slowly over the different fetuses. This is the main reason why these viruses in general do not cause abortion in sows, except if the virus infects several fetuses at once. The clinical outcome for these viruses in the fetus depends on the stage of gestation (Pensaert et al., 2004; Proszczy et al., 1980; Dunne et al., 1965; Koenen et al., 1994). Before the fetus can react with an immune response (± 70 days of gestation; ± 17 cm), infections are generalized and the fetus dies. Later on, the fetus may defend itself against the virus. The older the fetus is at the moment of infection, the better the survival rate. The problems are observed at birth: mummies of different size, still- and weakborn piglets. Besides these affected piglets, normal piglets may be born (uninfected or infected with immunity). Typical for PCV2 and EMCV are the high virus replication in the cardiomyocytes and the typical lesions in the heart (myocarditis).

Transplacental spread of PRRSV is mainly occurring after 70 days of gestation (Christianson et al., 1993; Terpstra et al., 1991). The reason for this is not clear. Karniychuk et al. (2009) quantified the macrophages carrying the receptor of PRRSV, sialoadhesin, and the entry mediator CD163. Both proteins are extremely important for the susceptibility of the macrophage for PRRSV. At the level of the uterine mucosa, a large concentration of sialoadhesin/CD163 double positive cells were found during the whole gestation period. At the level of the fetal placenta, double positive macrophages were found in the beginning and at the end of gestation. However, during mid-gestation the macrophages were carrying CD163 but lacked sialoadhesin. This type of macrophages is known to be refractory to infection. This could explain the lack of transplacental spread during mid-gestation. Replication of PRRSV is selective in macrophages in endometrium, fetal placenta and fetal organs. This does not lead to a quick mortality of the fetus. Because uterine macrophages are believed to play a role in tolerance to paternally-inherited antigens it is very well possible that destruction of these cells may lead to rejection of the fetus. This agrees with the symptoms in sows: late abortion/early farrowing. Research is ongoing in the author's laboratory to study this pathogenetic aspect. Partially mummified fetuses and stillborn piglets with edema are present.

The following approach may be used for the diagnosis of viral reproductive failure.

Because in general not all fetuses are infected, it is important to analyze whole litters of different sows.

A. Main complaint: abortion at different stages of gestation. When the fetuses are all fresh and firm (rigor mortis), then think in the direction of pathogens that cause general disease, such as SIV. Diagnosis should be done on the sow (virus isolation/PCR-nasal swab, seroconversion). When most fetuses are not fresh and some fetuses are partially mummified (brown) then suspect ADV or CSFV. Both viruses are eradicated, thus vigilance is extremely important. Typical for ADV are the necrotic foci on the surface of the fetal liver. Diagnosis should be performed in central veterinary institutes (immunofluorescence/virus isolation/PCR-lungs/spleen from fetuses). It is impossible to demonstrate a seroconversion because the sows have already seroconverted at the moment of abortion.

B. Main complaint: SMEDI - Stillbirth, Mummification at birth, Embryonic Death and Infertility. This syndrome is indicative for PPV, PCV2 and PEV. With EMCV stillbirth is the most important sign. In farms with gilts and sows that are correctly vaccinated against PPV, PPV can be excluded. PCV2 only poses a problem when the gilts are coming from a farm with a high health status (seronegative). Therefore, regular serological testing of gilts should be advised. PEV is not a problem in enzootic regions. EMCV is very rare and is normally linked with a plague of rodents. Diagnosis can be done by (i) virus isolation/PCR-heart/spleen/lungs from mummies or stillborn piglets (<17cm) or (ii) detection of antibodies in body fluids/serum (>17cm). Also for these viruses it is impossible to demonstrate a seroconversion because the sows have already seroconverted before parturition.

C. Main complaint: late abortion/early farrowing. This is typical for PRRSV. PRRS can be diagnosed by virus isolation/PCR-placenta/umbilical cord/lungs/spleen from partially mummified viruses/stillborn piglets. Antibodies are mostly absent in fetuses and stillborn piglets. Piglets that are normal at birth may be viremic.

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NON-INFECTIOUS REPRODUCTIVE PROBLEMS IN THE SOW: AN OVERVIEW

D Maes

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Introduction

Good reproductive performance is a prerequisite for pig herds to be profitable. Reproduction however is a complex process in which many different biological functions are involved, and in which many of the elements are interrelated. In case of reproductive failure in pig herds, it is therefore not always easy to determine precisely where failure has occurred. Reproductive failure or failure to achieve good reproductive performance can be grouped arbitrarily in six different categories related to the stage in the reproductive cycle namely anoestrus, ovulation and egg production, fertilization, implantation, fetal death and the mummified pig, and stillborn piglets. In most cases, failure can be narrowed down to one or two of these. Once the stage of reproductive failure has been identified then the causes or the risk factors for that failure should be found. Apart from infectious causes, there are many non-infectious factors related to management, nutrition and housing that may influence reproductive performance. It is a difficult but at the same time also a challenging task for the pig veterinarian to identify the most important risk factors in a problem herd. In addition, it is very rewarding for the veterinarian if he or she can solve problems that have lasted for a long time and have incurred major losses to a particular pig herd.

The present paper shortly reviews the most important non-infectious factors involved in reproductive failure or suboptimal reproductive performance in pig herds. Emphasis is placed on reproductive performance after weaning, repeat breeding, fetal death and stillborn piglets. Finally, the importance of a reliable record system is discussed.

Reproductive performance after weaning and wean-to-estrus interval (WEI)

During lactation, suckling suppresses estrus by preventing the release of GnRH from the hypothalamus. The act of weaning allows the release of FSH and LH, and the sow will normally show estrus within 4-7 days. The major challenge is to have sows coming into estrus as soon as possible post-weaning, and to keep the variability in onset of estrus between sows as low as possible. In addition, estrus signs must be clearly visible and a large number of good quality follicles should ovulate. Many different non-infectious factors such as length of lactation, body condition, season, and weaning management practices may influence one or more of these parameters (Koketsu and Dial, 1997).

WEI

When the WEI increased from 4 to 7 days, Steverink *et al.* (1999) found a decrease in farrowing rate (from 88% to 59% with a WEI of 9 to 12 days) and in litter size (from 11.7 to 10.6 piglets), respectively. This decrease was accompanied by a decrease in duration of estrus and a decrease in insemination-ovulation interval. The highest fertilization results were found when sows were inseminated between 0h and 24h before ovulation (Kemp and Soede, 1996). Therefore, the origin for the decrease in reproduction results could be found in the timing of insemination relative to ovulation. A decrease in ovulation rate could be a cause for the decrease in litter size with an increasing WEI. This is seen in several studies, where the ovulation rate decreased from 21.6 to 19.7 oocytes, when WEI increased from 3 to 6 days (Soede *et al.*, 1995 a,b; Steverink *et al.*, 1999). Aherne and Williams (1992) suggested that sows with a weight of 150kg or more at weaning, have minimum weaning-to-insemination intervals.

Lactation length

Data show that WEI increased rapidly as lactation length was reduced below 17 days, but WEI was relatively unaffected by lactation lengths of 17 to 30 days (Xue *et al.*, 1993). These data also emphasized that the percentage of sows bred by 6 days after weaning was significantly reduced for lactation lengths of 20 days or less. Koketsu *et al.* (1997) also reported lower farrowing rates if lactations are shorter than 3 weeks (17-19 days),

compared to sows weaned at 25 or 27 days. The sows with the short lactations also have higher risk to return to service, than those with longer lactations, indicating that lactation length has an additional effect on reproductive performance other than those mediated by a longer WEI (Vargas *et al.*, 2009a). As a minimum lactation period of 3 weeks is compulsory in the EU (Commission Directive 2001/93/EC of 9 November 2001), the possible negative influence of a short lactation length is less important than in countries where early weaning (<21 days) is widely practiced.

Clowes *et al.* (2003) however indicated that also extended lactations may negatively influence fertility post weaning, because it can cause catabolism of the sow body tissues. This may negatively influence the WEI, the quality of the follicles, the ovulation number and litter size in the next farrowing. This phenomenon of reduced fertility post weaning and lower litter size in the next cycle is well known in primiparous sows, and referred to as “second litter syndrome” (see further). Therefore, depending on the duration of lactation, the fertility of weaned primiparous sows may be affected by different mechanisms. To optimize management strategies, it is important to understand the cause of the variable (in)fertility with different lactation durations. If good breeding practices are applied, there is no difference on fertilization, return to estrus or the ovulatory process due to lactational effects (Willis *et al.*, 2003).

Body condition and feeding strategies

An inadequate nutrient and energy intake during lactation will also result in extended WEI, lower percentage of sows in estrus within 7 days of weaning, reduced pregnancy rate, and reduced embryo survival (Quesnel *et al.*, 1998). Management of the gilts is a very important starting point. Aherne and Williams (1992) reported that if gilts are inseminated at their second estrus, when they weigh at least 120 kg and have 17 to 20 mm back fat, less reproduction problems may be expected.

Primiparous sows need extra nutrients for growth, they have a lower feed intake capacity than older sows and they lack substantial reserves of fat and protein (ten Napel *et al.*, 1995). Therefore, they generally lose more bodyweight during lactation, what makes them more susceptible to impaired reproductive performance. Suboptimal reproduction of these sows post-weaning and in the next litter commonly occur in many pig herds, and referred to as the “second litter syndrome” (Morrow *et al.*, 1992). Koketsu *et al.* (1997) reported that a sow is 0.84 times less likely to have a regular return to estrus for each kg of increase in average daily feed intake. Different patterns of feed restriction in primiparous, lactating sows caused differences 1) in ovulation rate and embryo survival (Zak *et al.*, 1997a), and also 2) in the size of the follicles in the preovulatory pool and the maturation rate of oocytes obtained from these follicles (Zak *et al.* (1997b). Sows can sustain a loss of 9 to 12% of their body protein mass without adverse effects on piglet growth or ovarian function (Clowes *et al.*, 2003). However, if muscle protein loss in lactation exceeds 11 to 12% of the body protein mass at parturition, reproductive function is impaired. Vargas *et al.* (2009a) showed that loss of body condition during lactation was associated with return to service. Both primiparous sows and sows of second parity showed a higher risk of return to estrus if the body condition score dropped with more than 0.5 unit during lactation. This finding shows that even parity 2 sows can suffer from the negative effects of higher lactation losses on conception rate.

All these studies point to the fact that excessive body weight loss during lactation could reduce the ovulation rate, reduce oocyte and embryo quality, and ultimately lead to more repeats and lower litter size (Vargas *et al.*, 2009a). Feeding strategies should therefore aim to minimize weight loss during lactation and keep a sow’s body condition throughout her reproductive life. Apart from the beneficial effects on reproduction (Whittemore, 1996; Maes *et al.*, 2004), a good body condition is also important for the longevity of the sow and her well being.

As stated before, the issue of body weight loss is particularly important for primiparous sows, which represent 15% to 23% of the sow population of a pig herd with an optimal parity distribution. Sufficient feed intake in lactation can only be accomplished in case of a good appetite during lactation. Following factors should be considered: a good body condition at the time of parturition, an optimal stable climate (*e.g.* temperature) in the farrowing unit, a gradual increase of feed intake post farrowing, offering tasty feed several times per day, and providing sufficient drinking water that is easily accessible (nipple flow of at least 2 liters per minute). Another possibility is to allow the sow more time to regain a decent condition after weaning. This is possible by skipping a heat. Unfortunately, this leads to a significant increase in the number of non-productive days. Also altrenogest, a synthetic progestagen which physiological effects are similar to the sows own progesterone, can be used. By administering 20 mg a day, the reproductive cycle of the sow is blocked at the end of the luteal phase. Primiparous sows can be weaned 3-5 days earlier than older sows, and receive a post-weaning treatment

(Everaert *et al.*, 2007). Limiting the number of piglets for the first parity sows, split-weaning or intermittent suckling strategies could also be an option to limit body weight loss.

Season

Late summer and early autumn are the seasons when the reproductive parameters (onset of puberty, WEI, farrowing rate) consistently show the lowest values (Peltoniemi *et al.*, 1999). The seasonal infertility period of the domestic sow coincides with the non-breeding season in the European wild boar. The ancestral wild pig is a short day length seasonal breeder.

Weaning management and estrus detection

During the interval from weaning to insemination, sows can be fed to appetite. They are preferably housed in a dry environment with sufficient light *e.g.* 16 hours per day. Light intensity experienced by the sow can be affected by a number of environmental inadequacies *e.g.* too few or inappropriate height of the lighting, fly feces and dust on lamps gradually reduce the availability of light, or high walls surrounding animals or automatic feeders in front of sows producing shadows (Muirhead and Alexander, 1997; Tast *et al.*, 2005). One should be able to read the newspaper in the darkest parts of the building at sow level. Boar contact is very important to stimulate onset of estrus. If possible, sows should be moved to the boar for contact *e.g.* twice a day for approximately 20 minutes. In many herds, the boar is moved to the gilts because this is more practical. To achieve good reproduction results, it is also important to maintain a balanced parity distribution of the sows, and to avoid having too many young or old sows.

Detection of estrus is easier if the sow's behavior is observed in the presence of a boar, particularly when there is physical contact between the boar and the female. Management factors that interfere with the detection of estrus include housing submissive sows in groups with dominant sows, attempting to detect estrous females in large groups, and assessing estrus without using boar exposure.

Regular returns to estrus (day 18-24)

Sows, returning to estrus at regular intervals are likely to have experienced either conception failure or embryonic death of a critical portion of the litter before onset of implantation, such that pregnancy could not be maintained. Four embryos are required at nidation for pregnancy to be initiated. Otherwise, the sow will resume her cyclicity and a regular return to estrus will be observed. To solve problems with regular returns, special attention should be paid to 1) the semen quality and 2) the AI procedures. Semen quality should be assessed by evaluating the motility and morphology. Criteria for semen quality have been reviewed by Vyt (2007). For conventional AI, the insemination dose should contain at least 2 billion spermatozoa in 80-100 ml dilution solution.

Regarding AI procedures, especially the timing of insemination relative to the time of ovulation is critical. Estrus duration varies largely among sows (from 24h to 96h) and the time of ovulation depends on the length of this period (Soede *et al.*, 1995a). Female pigs ovulate at approximately two-thirds of the way through estrus. The ideal insemination time is 0-24 hours before ovulation, then fertilization results are higher than 90% (Nissen *et al.*, 1997). Knowing this, it is not surprising that many sows are inseminated too early or too late. Due to the limited lifespan of oocytes following ovulation and the long path spermatozoa have to travel before reaching the place of fertilization (Soede *et al.*, 1995b), the percentage of pregnancy could be very low and the number of returns to estrus may be high. Fertilization results (farrowing rate, litter size) drop quickly when insemination takes place after ovulation (Soede *et al.*, 1995a). In the study of Steverink *et al.* (1999) the farrowing rate decreased from 85% to 60% and the litter size from 11.5 to 10.8 piglets when insemination was performed 12h after ovulation instead of 28h before. In addition to reduced fertility, sows inseminated too late are also more susceptible to endometritis, compared to sows inseminated shortly before ovulation (De Winter *et al.*, 1992; Kemp *et al.*, 1998). Late inseminations, especially if done under poor hygiene conditions significantly increase the risk for the vulvar discharge syndrome.

Farms with a higher average farrowing rate and litter size had a longer duration of estrus. Estrus duration is influenced by factors such as parity, stress, boar effects and WEI (Kemp and Soede, 1996). Steverink *et al.* (1999) showed however, that the duration of estrus was not related to farrowing rate or litter size in individual pigs, but that there could be an indirect influence, via the chance to inseminate a sow or gilt at least once within the optimal period before ovulation. The number of inseminations per estrus, time of insemination and the total

duration of estrus were associated with each other, which make it difficult to detect which of these factors related primarily to farrowing rate or litter size.

Steeverink *et al.* (1999) also showed that the average duration of estrus on farms was consistent from month to month, with a repeatability of 86%, but that it differed between gilts and sows and between first estrus and repeat-breeders, and that it was affected by WEI. Gilts and repeat-breeders on average had a shorter duration of estrus than sows bred at the first estrus after weaning (Nissen *et al.*, 1997; Steeverink *et al.* 1999). Most likely, they have more chance to receive post-ovulatory inseminations, mainly if the onset of estrus is incorrectly detected (Vargas *et al.*, 2009a). Variation between farms could be explained by the above specific factors that may influence the total duration of the estrus: stress conditions, differences in the quality of an individual boar, breed, or, possibly, nutritional condition of sows and gilts. Based on these results, it appeared that farmers can predict the average duration of estrus in gilts and sows, based on the information of the former month. Consequently, they can estimate the right insemination time, based on the predicted duration of estrus and the knowledge that ovulation takes place two-thirds of the way through estrus (Nissen *et al.*, 1997).

Apart from semen quality and timing of AI, also many other factors are associated with an increase in occurrence of return to service (Table 1). These include gilts and primiparous sows, inseminations during warm months, short lactation lengths, low feed intake during lactation, poor environmental conditions (draughts, darkness), stress during the first 12 days after AI, etc. (Koketsu *et al.*, 1997). A high level of feeding during early pregnancy, especially in moderately prolific gilts, has been associated with a reduction in embryonic survival in some former studies. However, a recent study (Quesnel *et al.*, 2009) showed that a high feeding level (4 kg vs. 2 kg gestation feed per day) for prolific gilts did not reduce embryo survival, and had no beneficial nor detrimental effects on embryo size and variability at 27 days of gestation.

Sows inseminated in the second estrus after weaning, have mostly a larger litter size than those mated in the first estrus after weaning (Santos *et al.*, 2004). The longer the period is between the parturitions, the better the body condition score and metabolic status is in repeat breeders. This is mainly important for the primiparous sows, who suffer from the consequences of the lactational catabolism (Tummaruk *et al.*, 2001). In a study of Vargas *et al.* (2009b) however, the smaller litter size of re-serviced gilts was probably not a nutritional matter.

Irregular returns to estrus (>24 days)

Females, returning at irregular intervals likely conceive but lose their litters after the onset of implantation, but before fetal calcification (35 days), leaving incompletely resolved embryonic remnants that delay the luteolysis of the corpora lutea and delay the resumption of the ovarian cyclicity (Dial *et al.*, 1992). In many herds, approximately two thirds of the sows returning to estrus are regular returns, and one third irregular returns. This may depend on the herd and/or the study. In a study of Koketsu *et al.* (1997), the proportions of regular and irregular returns to service were 44% and 56%, respectively.

Boar factors are usually of low priority for irregular returns because the failures are much more likely to be of a maternal nature. The same factors mentioned for regular returns may also apply here. Infections become more important and any (subclinical) disease of the sow could result in embryo mortality and irregular return to estrus. Sows with ovarian cysts have a greater return to estrus rate than normal sows (34 vs. 8%) (Castagna *et al.*, 2004). Most of the sows with multiple large cysts show intermittent or permanent anoestrus. In contrast, multiple small cysts often produce estrogen, and sows may have irregular estrous cycles. Consumption of grains containing the estrogenic mycotoxin, zearalenone, may result in irregular return to estrus, small litter size and increased stillbirth rates.

Table 1: Main factors involved in regular and irregular returns to estrus

Regular returns	Irregular returns
Boar / semen	Disease
Management	Management
Lactation length	Environment
Wean to estrus interval	Season
Environment	Other (mycotoxins, ovarian cysts)
Parity	
Disease	

Fetal death and the mummified pig

At approximately 35 days of gestation, the embryonic phase ends and the fetal phase begins. In the pig, this is also the period in which skeletal calcification begins. This means that if a piglet dies, it is not completely absorbed and a mummified fetus remains. Mummies are fetuses that have died *in utero* and have begun to decompose. There are two possible causes of mummies. First, a piglet dies because there is a large litter and insufficient space in the uterus. Second, there is infectious disease. In the first case, a study of the records will show that the mummified pigs are occurring in large normal litters. For example, a litter size of 14 alive, one dead, one mummified is of no significance, provided the remainder of the litter is normal and healthy. In case of disease, mostly the number of liveborn piglets is too low and piglets may not be healthy, and there are more mummified pigs (Muirhead and Alexander, 1997).

Dead fetuses can undergo mummification, but also abortion. Abortion means the premature expulsion of a dead or non-viable fetus between D35 and D109 of gestation. Survival before day 109 of gestational age is limited because the lungs are not fully mature by this age. Abortion results from the termination of the pregnancy control mechanisms with subsequent expulsion of all conceptuses. As the corpora lutea are necessary until the end of gestation, any factor that induces luteolysis will cause abortion. Aborted sows return to estrus within 5-10 days or experience a prolonged anestrus.

Non infectious causes of abortion in the sow include aberrant temperature, season, toxic substances, mycotoxins, excessive negative energy balance and stress. Extremely high or low temperatures *e.g.* due to inappropriate housing and ventilation, may cause abortion. Depending on the housing conditions, pregnant sows are housed at approximately 18°C. The risk for abortion is higher for sows mated in late summer ("seasonal abortion"). This is particularly evident in outdoor sows where pregnancy failure may reach 15-30%, and in group housing systems. High levels of carbon monoxide (CO) in underventilated facilities may cause fetal death. The sows remain healthy because the fetal hemoglobin has a higher affinity for CO than the sow hemoglobin. Inappropriate treatment (too frequent, overdose) with toxic substances such as organic phosphorous, may cause abortions and stillborn piglets. Also, injections with procain penicillin, especially in the beginning of gestation, may cause embryonic mortality and abortion (Embrechts, 1982). Feed contaminated with mycotoxins such as *Claviceps purpurea*, T2-toxin en zearalenone, may lead to abortion, stillborn and weakborn piglets (Osweiler, 2006). Mycotoxicosis can be diagnosed by analyzing the feed for presence of mycotoxins. Excessive negative energy balance may lead to abortion.

Stress in pigs can be caused by very different factors such as injections, reaction to vaccination, discomfort by fighting for social ranking (*e.g.* mixing sows ,overcrowding), poor stable climate (draughts, too cold or hot) and inappropriate feeding. Many studies have shown that stress may negatively influence reproductive performance (Almond, 1992; Martineau, 1997). Stress is particularly harmful during the first weeks of gestation (during migration and implantation of the embryos). Stress can also be harmful later on during gestation, but a more severe stress reaction is needed for inducing abortion (>35 days of gestation) than for inducing embryonic mortality (<35 days). The precise mechanism of the negative influence is unknown, but possible factors like an increase in body temperature and/or increased contractility of the uterus caused by excitation may play a role. The effects are more severe in stress-sensitive breeds (Piétrain) or lines than in other breeds (*e.g.* commercial hybrid sows).

Stillborn piglets

Pig producers and breeders have made major efforts to improve sow productivity through genetic selection for increased litter size. However, concurrently with the selection for litter size, also the number of stillborn piglets has increased, limiting the overall effectiveness of selection for increased litter size (Canario *et al.*, 2006; Distl 2007; Rosendo *et al.*, 2007). Reported stillbirth rates vary between 3 and 8 % (Cutler *et al.*, 2006). Of all stillborn piglets, 10% dies shortly before farrowing, 75% during farrowing and the remaining 15% immediately after farrowing (Leenhouders *et al.*, 1999). Pre-partum deaths are usually due to infections whereas intra-partum deaths are more associated with non-infectious causes (Alonso-Spilsbury *et al.*, 2004). Birth asphyxia is the major cause of the immediate natal and early postnatal losses (Van Dijk, 2008).

Several factors have been associated with the occurrence of stillbirths. Some of them are associated with sow characteristics such as breed, litter size, parity, sow condition, gestation length, farrowing duration (Leenhouders *et al.*, 1999; Canario *et al.*, 2006; Taverne and van der Weijden, 2008; Vanderhaeghe *et al.*, 2009). Others are associated with piglet characteristics such as birth interval, birth order, piglet haemoglobins

and piglet birth weight (Leenhouders *et al.* 1999, Canario *et al.*, 2006) or management, housing and feeding practices. Different factors may be interrelated. An increased farrowing duration has been associated with increased litter size, shorter birth interval, lower birth weights, shorter gestation length, increasing parity, and greater need for birth assistance (Holm *et al.*, 2004). Data on the impact of management related factors on stillborn piglets are scarce and mainly focus on supervision of farrowing (Lucia *et al.*, 2002), pen design (Fraser *et al.*, 1997), ambient temperature (Odehmalova *et al.*, 2008) or dietary fibre (Guillemet *et al.*, 2007). Diets deficient in vitamin A, zinc, copper and iodine during gestation or containing mycotoxins were all reported to increase the incidence of stillbirth.

Record system and targets for performance parameters

It is obvious that assessing the stage of reproductive failure and identifying the associated risk factors is not always straightforward. As there are numerous risk factors or differential diagnoses for the different types of reproductive failure, a diagnostic examination of the environment, management, nutrition, housing, infectious diseases is necessary. However, a reliable record system that allows monitoring the reproductive performance of the sows is extremely helpful to explore the problem, and is a *conditio sine qua non* in most pig herds. To understand and interpret records, universally accepted definitions for analyzed parameters have been developed (Almond *et al.*, 2006). The most important ones are (adjusted) farrowing rate, conception and farrowing rate, regular and irregular returns to estrus, litters/sow/year, total number of pigs/litter, pigs weaned/sow/year, nonproductive days, mummies and stillborn pigs, and WEI. Numerous systems are currently available for assessing the reproductive performance of the breeding herd. Although they vary considerably in data entry, report format and report content, all systems provide summaries of breeding, farrowing and weaning information (Dial, 1990). Targets and level at which a corrective intervention should be performed (interference levels) should be included in these production reports (Table 2). The values of these parameters need to be changed regularly as the herd performance change.

The most widely used measure of the overall reproductive performance of the breeding herd is pigs weaned/sow/year (Polson *et al.*, 1990a). This measure is composed of two components namely litters/sow/year and number of pigs weaned/litter.

Table 2: Targets for the reproductive performance of the breeding herd
(adapted from Almond *et al.*, 2006)

	Target	Interference level
Breeding and Gestation		
Age at first service (days)	220-240	<220 or >260
Repeat matings (%)	10	>15
Multiple matings (%)	90	<85
Weaning to service interval (days)	4-7	>7
Farrowing rate (%)	≥85	<80
regular returns (%)	<6	>8
irregular returns (%)	<3	>5
negative pregnancy test (%)	<3	>5
abortions (%)	<2	>4
failure to farrow (%)	<1	>3
Adjusted farrowing rate (%)	≥90	≤88
Farrowing		
Total pigs born/litter	≥11.5	<11
Pigs born alive/litter	≥10.5	<10
% stillbirths	<7	>10
% mummies	<3	>5
Litters/productive sow/year	>2.4	<2.3
Litters/sow/year	>2.2	<2.1
Weaning		
Pigs weaned/sow	≥10	≤9.8
Pre-weaning mortality (%)	<8	>10
Pigs weaned/productive sow/year	>24	<23
Pigs weaned/sow/year	>22	<21
Population (on an annual basis)		
Average parity	3.5	<3 and >4
Replacement rate (%)	≤40	<35 and >45
Culling rate (%)	30-35	<28 and >40
Mortality rate (%)	5-8	>10
Average non productive days (60d acclimatization period)	≤75	>80

Conclusions

Reproductive failure can occur at one or more stages of the reproductive cycle. Once the stage of reproductive failure has been identified then the causes or the risk factors for that failure should be identified. A structured record analysis in conjunction with the use of flow diagrams that indicate the different factors and their level of importance is very helpful to understand and solve problems of suboptimal reproductive performance. An organized system is also less time-consuming and more efficient. This system should also allow validation of progress and assessment of response to management changes in order to obtain maximal reproductive efficiency of the breeding herd.

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PORCINE ULCERATIVE DERMATITIS SYNDROME (PUDS): A FORM OF VESICULAR CUTANEOUS LUPUS ERYTHEMATOSUS (VCLE) IN SOWS?

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Summary

Ulcerative skin lesions in sows are only poorly documented in literature, likely because of the low economical importance that is given to these conditions. The present study reports two cases of severe ulcerative skin lesions in sows diagnosed as porcine ulcerative dermatitis syndrome (PUDS). Different clinical, pathological and immunological parameters were measured for the diagnosis and to get more insight into the possible aetiology of PUDS. Severe ulcerative lesions were observed in the skin of two sows in a farrow to finish pig herd comprising 540 hybrid sows. Annular to polycyclic dermal severe crustaceous ulcerations were located at the abdomen and flanks. Moderate lesions were also found at the base of the tail and the perineum. The lesions were histologically characterized as a cell-poor interface dermatitis and folliculitis with basal cell vacuolization, vesicle formation at the dermal-epidermal junction and serocellular crusts. A subepidermal mild to moderate band characterized as a mixed inflammatory infiltrate was present. Antinuclear antibodies (ANA) test was negative; however immunofluorescence testing revealed a linear pattern of IgG precipitation in the skin. *Staphylococcus hyicus* was demonstrated in the serocellular crusts of one sow. Porcine circovirus and porcine respiratory and reproductive syndrome virus could not be isolated from necropsy samples. Treatment with antibiotics, topical antiseptics and corticosteroids did not improve the condition. Although antibodies to Ro/SSA and La/SSB were not examined, the histological lesions and the presence of immunoglobulin deposits in the skin are suggestive for an autoimmune dysfunction, resembling vesicular cutaneous lupus erythematosus (VCLE). In conclusion, the clinical symptoms and the severe gross lesions observed in the two sows were characteristic for PUDS. Based on the histological findings and the immunostainings, the lesions were compatible with vesicular cutaneous lupus erythematosus (VCLE) as described in humans and Shetland sheepdogs and collies. Immune-suppressive therapy in swine herds can not be justified from an economic point of view. As the condition hampers animal welfare, affected pigs should be humanely euthanized.

ASSOCIATION BETWEEN DETECTION OF HAEMOPHILUS PARASUIS, MYCOPLASMA HYORHINIS AND THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN PIGS

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Objective

The objective of this study was to determine the associations between the detection of *Haemophilus (H.) parasuis*, *Mycoplasma (M.) hyorhinis* and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in pigs with polyserositis.

Material and methods

A total of 143 animals from 72 farms were included in the study. All pathogens were detected either by polymerase chain reaction (PCR) investigation of swabs of the serosal surfaces for *H. parasuis* and *M. hyorhinis*, or PCR investigation of lung tissues for PRRSV (EU-field strain).

Results

A significant association between the detection of *H. parasuis* and *M. hyorhinis* was detected (Odds-Ratio: 4.59; $p < 0.001$). Pigs that tested positive for PRRSV (EU-field strain) were significantly more often positive for *H. parasuis* (Odds-Ratio: 4.32; $p = 0.014$) and for *M. hyorhinis* (Odds-Ratio: 5.206; $p = 0.005$). The results of this study indicate that pigs, which are already infected with PRRSV (EU-field strain), have a higher risk of infection of the serosal surfaces with *H. parasuis* and *M. hyorhinis*. Furthermore, it was possible to show that pigs that have a polyserositis often have co-infections with *H. parasuis* and *M. hyorhinis*.

Discussion and conclusion

Kobayashi et al. (1996) were able to isolate *H. parasuis* and *M. hyorhinis* from the same individual pigs. A possible explanation for these results is offered by the affinity of both pathogens to serous membranes. However it is not known to what extent these pathogens interact with each other or whether the presence of one acts as a trigger for the other. The association between infection with PRRSV and the occurrence of bacterial infections has been examined by various authors. Solano-Aguilar et al. (1997) described the detrimental influence of a simultaneous infection with PRRSV and *H. parasuis* on the mortality of piglets. However the results of a study performed by Cooper et al. (1995) in which SPF-piglets were infected with PRRSV and one week later with *H. parasuis*, were not concordant with these results. The authors reported that no differences between the group solely infected with PRRSV and the group infected with both pathogens occurred. Segalés et al. (1999) also could not find any influence of a previous infection with PRRSV on the appearance of Glaesser's disease. The present study supports the assumption that a simultaneous infection with PRRSV and *H. parasuis* or *M. hyorhinis* occurs significantly more frequently than single infections with individual pathogens. According to Shimizu et al. (1994) and Kawashima et al. (1996), a simultaneous infection with *M. hyorhinis* and PRRSV causes severe pneumonia, whereas a mono-infection with PRRSV only causes mild pneumonia. In the present study, an association between positive results for PRRSV and *H. parasuis* or for PRRSV and *M. hyorhinis* could be detected. It is possible, that PRRSV damages lung-tissue in a way that makes it easier for pathogens that frequently occur in lungs of pigs, to overcome the lung's physiological barrier and colonise serous membranes.

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DIAGNOSTIC EXAMINATION OF INCREASED SOW MORTALITY IN BELGIAN PIG FARMS

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Introduction and Objective

Sow mortality is one of the most important economic losses in sow herds. A previous study showed that sow mortality in Flanders was about 3.5% yearly (1). Establishing a diagnosis of sow mortality is often difficult, and therefore data on causes of sow mortality in Belgium are scarce. The present study aimed to investigate the causes of sow mortality in 10 sow herds in Belgium suffering from an increased sow mortality (>10% on annual basis).

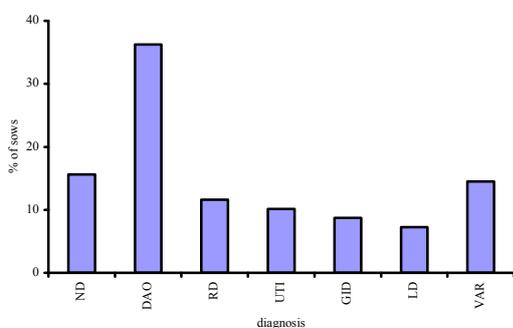
Materials and Methods

Sow herds (n = 10) were selected on the basis of a sow mortality of at least 10% on annual basis. All dead sows were necropsied at the laboratory of Animal Health Care Flanders during (specifieke periode aangeven)

Results

Studying total, 69 sows were necropsied, with a minimum of 2 and a maximum of 13 per farm. The main diagnoses are shown in Figure 1.

Figure 1. Percentage of main diagnoses of the necropsied sows (n=69). ND, no diagnosis; DAO, displaced abdominal organs; RD, reproductive disorders; UTI, urinary tract infections; GID, gastro-intestinal disorders; LD, locomotive disorders;



; VAR, various other diagnoses

Table 1. Farm-specific diagnoses with number of necropsied sows and final solution of the sow mortality problem

Farm	Farm-specific diagnosis	Nb of cases	Solution
1	Gastric ulcers	2	yes
2	Gastric ulcers & spleen torsion	6	yes
3	Gastric ulcers & displacement	7	yes
4	Spleen torsions	3	yes
5	Liver lob torsion	8	yes
6	Urinary tract infections	7	yes
7	Variable ... mycotoxins	13	yes
8	Variable ... mycotoxins	13	yes
9	Locomotive disorders	5	no
10	No diagnosis	4	no

In Table 1, diagnoses are grouped per farm. From these results, it was obvious that 3 farms (N°1-3) had a problem of gastro-intestinal disorders, mainly gastric ulcers, in combination with displaced abdominal organs. Specific adaptations in feeding strategy and coarseness of the feed could solve the problem rather quickly. Farm N°4 and 5 had a problem with displaced abdominal organs, namely spleen and liver lobes, which could be solved through a shift in breeding stock origin. On farm N°6, urinary tract infections were the specific problem, which was related to poor water quality, due to heavy contamination of the water distribution pipes. Following a thorough cleaning procedure, the problem was solved. The clinical picture and necropsy data on farm N°7 and 8 were variable and unclear. Following additional analysis, a problem of mycotoxins could be identified. In farm N°7, farm-grown CCM was fed to the breeding stock. After addition of a mycotoxin binder to the feed, the problem was solved on this farm. Farm N°8 fed a soup mix, which was prepared in a mixing installation with low hygiene, to its breeding stock. Following a good cleaning procedure, sow mortality could be normalised. In only two farms (N°9 and 10), no clear diagnosis could be established and the problem of sow mortality remained unsolved.

Discussion and Conclusions

Compared to other studies on sow mortality, a rather small number of animals were necropsied in the present study (2,3,4). Nevertheless, similar results of causes of sow mortality were obtained as in other recent studies (3). Displacement of abdominal organs was observed as the most important cause of death (2,3), whereas urinary tract infections and disorders related to the reproductive system were the second most prevalent causes (2,3). Even the level of unknown causes of death was within the expectations (15% vs. 21.8% (2)). In conclusion, systematic necropsy of dead sows is a very useful tool to establish a diagnosis at herd level and subsequently to solve problems of increased sow mortality.

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OCCURRENCE OF *LAWSONIA INTRACELLULARIS*, *BRACHYSPIRA HYODYSENTERIAE* AND *BRACHYSPIRA PILOSICOLI* AND THEIR RISK FACTORS IN SWITZERLAND

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Objective

Porcine proliferative enteropathy (PPE), swine dysentery (SD) and porcine intestinal spirochaetosis (PIS) are widespread enteric diseases caused by *Lawsonia intracellularis* (*LI*; PPE), *Brachyspira hyodysenteriae* (*BH*; SD) and *B. pilosicoli* (*BP*; PIS). Little is known about their epidemiology and significance in Switzerland. Especially SD and PIS were described as a suspicion and detected only by culture but never by PCR in Switzerland. The aim of this study was to determine the PCR-associated prevalence of these three diseases in Switzerland and to identify potential risk factors for positivity at the herd level.

Materials and methods

A total of 122 pig herds, 43 with diarrhea of unknown cause, 59 control herds without diarrhea and 20 free range farms were included. On each farm fecal samples from the rectum were collected from 5 animals of the same age (weaners or fattening pigs) and analysed by PCR for *LI*, *BH* and *BP*. A rectal swab from one of these pigs was tested for haemolytic *Escherichia coli* (*EC*). If bloody diarrhea was observed, faeces were also examined for *Salmonella* sp. by culture and parasitology. Every farmer was interviewed by the same person about factors such as feeding, stable conditions, management, number of animals and treatment. The hygiene and the climate was assessed by the interviewer. The association between potential risk factors and disease (PCR positivity) was assessed using cross tabulations with Fisher's exact tests and multivariable logistic regression models (NCSS).

Results

122 farms (617 samples) were examined. Results are shown in figure 1.

LI (103 out of 200 pigs) as well as *EC* (31) was detected in every group. Pigs from all farms positive for *BP* had symptoms of diarrhea. PCR prevalence on the 8 infected farms was over 40% (18/43). All of the 3 infected herds with *BH* had pigs with clinical symptoms, and PCR prevalence was almost 100% (14/15).

Double or triple infections were observed.

Risk factors for a herd to be positive for *LI* were > 300 animals, group size of 37-60 and >107 animals, >20% slotted floor, feed pipelines, feed administration 3 times or more/day and use of not *LI* effective antibiotics. Protective factors were a separation box and storage of feed in bags.

Risk factors for *BP* were several origins of piglets, wet litter, whey, feed pipelines, dirty alleys and other diseases. A ramp and dry litter were protective factors.

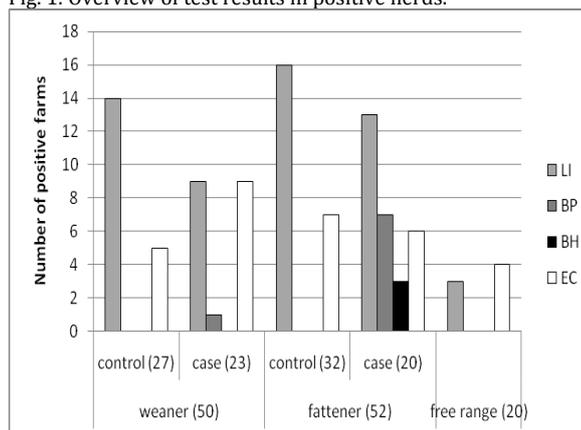
Discussion

For the first time *BH* and *BP* were detected by PCR in Switzerland. *LI* seems to be a more or less ubiquitous agent being detected in control herds even more often than in farms with diarrhea. Other factors such as e.g. *EC* are likely to cause enteric problems in these herds. *Brachyspira* infections, however, seem to be related to diarrhea and require adequate measures.

Conclusion

LI is widespread in Swiss pig herds, irrespective whether clinical signs occur or not. Diagnosis of PPE should be more differentiated than the detection of the bacterium alone. Detection of *Brachyspira* sp. requires medication or eradication programs. More investigations – in particular development of a herd monitoring system - are necessary.

Fig. 1: Overview of test results in positive herds.



Numbers in parentheses indicate the total number of herds in this group. *LI*, *BH* and *BP* were detected by PCR, *EC* by routine bacteriology.

AFRICAN SWINE FEVER OUTBREAKS CLOSE TO EU

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This talk will summarize the basic information on African Swine Fever (ASF) virus to support the subsequent description of recent African swine fever outbreaks close to EU. ASF is endemic at Sardinia and has caused occasional outbreaks in central Europe, such as 1985 in Belgium and 1999 in Portugal and Spain. In 2007 an undiagnosed disease in domestic pigs were observed in Georgia, but ASF was only suspected after the more obvious suspicions have been negative.

ASF is a controversial disease in pigs caused by a large double stranded DNA virus, belonging to the family *Asfiviridae*, as the only member. The virus is relatively stable in the environment especially if present in proteinaceous materials, such as dead animals. ASF virus only infect porcines, however the uncontrollable reservoir in wild boar and warthogs is of large concern for eradication of the disease in domestic pigs. The clinical picture of ASF is as for classical swine fever: high fever, bleedings in the skin and tissues, severe lymphoid depletion, central nervous symptoms and high mortality (Wardley et al., 1983). The virus causing classical swine fever is a single stranded RNA pestivirus very different in spite of the similarity in clinics. ASF virus may be transmitted directly from pig to pig or it may use the Sylvatic circle where the virus is arthropod borne, using a soft-tick vector *Ornithodoros sp.*. There are no vaccines against ASF and solid protection across genotypes is questionable.

The first positive diagnosis of ASF in Georgia was reported in June 2007 following months of undiagnosed mortality. At this time point 52 out of 65 districts were affected (Beltran-Alcrudo et al., 2008). In August ASF was detected in Armenia followed by the Russian Federation in December 2007 and Azerbaijan January 2008. Before March 2009 a total of six territories were infected. Until July no new cases have been detected in Russia increasing the hope that the disease is under control. However, as transmission in 2008 resulted in 1-7 months between cases it is still too early to ascertain that ASF is eradicated.

Shortly after the diagnosis an expert team from EU/FAO/OIE visited Georgia, resulting in a very depressing report where one of four suggestions was to kill all pigs in Georgia thereby hoping to control the disease. The majority of affected pigs in Georgia were free ranging complicating the control. Occasional pig carcasses were reported to be seen in the countryside. Transmission of ASF to wild boars assisted in effective transmission of ASF through the valleys northbound.

The introduction of virus into Caucasus is probably through kitchen waste from international ships embarking at Port of Poti. Sequence studies of the Caucasus ASF strain show similarities to ASFV in Southeast Africa, especially Madagascar and Zambia.

There is a lack of information on the distribution in Caucasus on *Ornithodoros* or other soft ticks that may harbour ASF. If the vector is present eradication will be extremely difficult as the tick will remain contagious for a new susceptible pig for several years (Basto et al., 2006).

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NEW NEONATAL DIARRHOEA SYNDROME IN DENMARK

B Svensmark

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The Laboratory of Swine Diseases, Kjellerup is operated by the Danish Agriculture and Food Council and performs diagnostic service for swine veterinary practitioners from all parts of Denmark. Each year 2-4000 pigs are submitted for post mortem from herds with disease problems. Routine pathological and microbiological examinations are carried out in Kjellerup, while specialized tests is carried out in collaboration with Technical University of Denmark, National Veterinary Institute.

In the past 3 years approximately 80% of laboratory submissions were due to clinical problems with neonatal diarrhoea. All submitted pigs were autopsied and examined microbiologically by routine methods. The typical pathological findings were often minor and non-specific since the pigs are without signs of dehydration and have a normal content of milk in the stomachs. The small intestines were usually either contracted or atonic and dilated. There was rarely hyperaemia or congestion in the intestines. Intestinal contents were yellowish and aqueous, the intestinal mucosa had no changes and the lymph nodes mostly without reaction. Likewise colon and caecum had a normal intestinal mucosa while the content was creamy to watery with a yellowish colour. Generally, no pathological changes were observed in organs other than intestines.

The routine examinations included aerobic cultivation of *E. coli* with subsequent serotyping and anaerobic cultivation for *Cl. perfringens* type A and C. Some of the pigs are also tested for *Cl. difficile* and rotavirus.

Results

Table 1. Bacteriological and virological findings in 220 submissions of pigs younger than 5 days with anamnesis of clinical signs of enteritis in 2008 and 2009

Agent	Positive	Total analysed	Percent positive analyses
<i>Non-hemolytic E. coli</i>	121	220	55
<i>Hemolytic E. coli</i>	15	220	7
<i>Enterotoxigenic E. coli</i>	32	195	16
<i>Cl. perfringens type A</i>	177	220	80
<i>Cl. perfringens type C</i>	1	220	0
<i>Cl. difficile</i>	20	63	32
Rotavirus	16	134	12

Table 2.

Distribution of serotypes in the 15 submission with hemolytic *E. coli* shown in Table 1.

<i>E. coli, serotype O8</i>	13
<i>E. coli, serotype O45</i>	1
<i>E. coli, serotype O149</i>	1

Discussion

The microbiological testing was in most cases not sufficient to explain the problem in herds with severe neonatal diarrhea. Also the histological studies of the intestines of pigs with acute diarrhoea were generally inconclusive.

It is note worthy that *E. coli* serotype O149 has virtually disappeared during recent years. This may be due to fact that genetic selection for F4 receptors has been carried out in the Danavl breeding herds since 2003 At present *E. coli* serotype O8 represent the predomination *E. coli* type in neonatal diarrhoea.

Non-hemolytic *E. Coli* and *Cl. perfringens* type A may be isolated in pigs without enteritis, and are therefore not considered to be important causal agents of for enteritis. The role of *Cl. difficile* in neonatal enteritis under Danish conditions is not adequately elucidated.

There is therefore a need for further studies of the causal nature of neonathal diarrhoea in pigs.

GASTRIC ULCERS IN PIGS: DO BACTERIA PLAY A ROLE?

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Introduction

Gastric ulcers are common in pigs worldwide. They are found in the proximal non-glandular region of the porcine stomach. This region and the cardiac gland zone have a pH range between 5 and 7 due to the presence of saliva and cardiac gland bicarbonate secretion. In the distal compartment, composed of the fundic and pyloric gland zone, hydrochloric acid is secreted resulting in a low pH. Ulceration in the non-glandular region of the porcine stomach is a disease of complex etiology in which multiple factors are involved, including dietary and stress factors. Small particle size of feed and interruption of feed intake promote ulcers. More generally, all conditions resulting in a breakdown of the pH gradient between the proximal and the distal parts of the stomach, such as increased fluidity of the stomach contents, may play a role in ulcer development (1). In the present paper, the role of bacteria in development of gastric ulcers in pigs is briefly discussed.

Bacteria associated with gastric ulcers

Helicobacter suis is a Gram-negative, very fastidious bacterium with a long spiral-shaped morphology, which is highly prevalent in pigs. Most probably, this agent is of zoonotic significance. It has been associated with gastritis, gastric ulcers and gastric MALT lymphoma in humans (2). *H. suis* is one of the factors playing a role in the pathogenesis of gastric ulcer disease in pigs, as was shown in a recent study. Nine 6-week-old piglets were intragastrically inoculated with a pure culture of *H. suis*, while 5 sham-inoculated piglets were used as controls. All piglets were fed a finely ground diet. Hyperkeratosis and ulcer formation were clearly present in the gastric non-glandular mucosa of all *H. suis* inoculated pigs, while none of the sham-inoculated piglets developed gastric lesions. *H. suis* colonizes mainly the pyloric and the fundic gland zone and has been found in close contact with parietal cells. An infection with this agent might result in secretion of excessive amounts of gastric acid, leading to increased contact of the non-glandular part of the stomach with hydrochloric acid (2).

Other bacteria that occasionally have been associated with gastric ulcers in pigs include a curve-shaped helicobacter morphologically different from *H. suis*. No genomic data of this bacterium have been published (2). Krakowka et al. (3) produced lesions of the non-glandular region of the stomach in gnotobiotic piglets fed a carbohydrate enriched diet and inoculated experimentally with *Lactobacillus* spp which were not identified to the species level. Infections of the respiratory tract have been associated with an increased likelihood of gastric ulceration. This may be due to inappetence or to release of histamine which may increase gastric acid production.

Conclusion

H. suis is the main bacterium playing a role in the development of hyperkeratosis and ulcer formation in the gastric non-glandular mucosa of pigs. Porcine gastric ulcer disease is, however, not of monocausal origin and several other factors are also involved.

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INFLUENZA A IN PIGS – IMPLICATIONS FOR ANIMAL AND HUMAN HEALTH

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Influenza A virus infects a number of species, including swine, humans and birds. In general, influenza A viruses are species specific, but there are several examples of viruses jumping from one species to another. Since pigs seem to have receptors for both avian and human influenza subtypes, pigs has been regarded as a possible mixing wessel for new influenza subtypes.

There are two important aspects linked to influenza A infections in pigs.

First, influenza A virus infections are a common cause of respiratory disease and abortions in swine. The impact on the production economic and animal welfare of influenza A are in our opinion underestimated. Some recent results of the impact of influenza A infections of piglets will be presented.

The latest outbreak of a new influenza A in humans (the so called swineinfluenza, Mexican flu or newH1N1) has sparked an increasing public focus on the zoonotic potential of swine influenza viruses which the pig producers and advisers have to deal with in the future. Many wrong interpretations have been publishes in various media on the origin and impact of this new virus in relation to pigs. Facts on the new influenza type, updated news on its distribution and results of recent experimental infection studies in pigs will be presented at the meeting as an introduction to a wider discussion on the subject – influenza A as a pig zoonosis.

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN PIGS AND IN OCCUPATIONALLY EXPOSED PERSONS

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Introduction/Objective

There are three main MRSA complexes which have to be differentiated. The first MRSA complex is the hospital-acquired MRSA (haMRSA) complex. Persons at risk are the so called YOPIS (young, old, pregnant, immune suppressed) who are patients or employees in hospitals or inhabitants or employees of healthcare facilities or elderly homes. The second MRSA complex is the community-acquired MRSA (caMRSA). Persons at risk are any healthy persons without specific risk factors. The third and newly established MRSA complex is the livestock-associated MRSA (laMRSA) complex, in which in more than 95% of all cases only one clonal line, the MRSA ST398, occurs. Since 2005, there have been reports from the Netherlands, Canada, Belgium and Germany about this special clonal MRSA line in pig herds. Apart from pigs, there are also reports on calves, cows and poultry. Persons at risk are persons occupationally exposed to livestock. The objective of the presented investigations is to increase the knowledge on the occurrence of laMRSA both in pigs and people that are exposed to pigs (veterinarians and meat inspectors).

Material and Methods

In three different studies investigations on the occurrence of MRSA in domestic pigs, wild boars and occupationally exposed persons to pigs were carried out as follows:

- a) MRSA as nasal colonizer of domestic pigs from conventional husbandry systems. Therefore we tested 687 pigs from 347 different farms and age groups, which were sent to the Field Station for Epidemiology for clarification of herd health problems by necropsies and laboratory diagnostics.
- b) MRSA as nasal colonizer of wild boars hunted in different parts of Germany. From 75 hunted wild boars nasal swabs were taken and cultured on MRSA-selective chromagar plates.
- c) MRSA as nasal colonizer of occupationally with pigs exposed persons. Nasal swabs were taken from 28 specialized pig practitioners, 8 employees of the Field Station for Epidemiology and from 50 meat inspectors in pig slaughterhouses.

Results

In study a) from the 678 tested pigs in 85 cases MRSA (a frequency of 13%) could be found as nasal colonizer. The MRSA isolates all were identified as belonging to the livestock-associated MRSA type ST398. As for b) all 75 tested nasal swabs from wild boars were MRSA-negative. Besides other bacteria like *Streptococcus* spp., Methicillin-sensitive *Staphylococcus aureus* (MSSA) was detected in 10 wild boars. Regarding the results of occupationally exposed persons, the highest frequency of MRSA was found in the group of the diagnosticians of the Field Station for Epidemiology (3/8; 38% MRSA-positive). In the group of pig practitioners 10 out of 28 tested veterinarians were MRSA-positive (36%) and 7 out of 50 tested meat inspectors (14%).

Discussion

In accordance with several similar studies in other countries, our results on the MRSA frequency in domestic pigs at the individual animal level seem to be the same. The occurrence of MRSA in wild boars in Germany is obviously very low (0/75). However, this cannot be attributed to a natural non-susceptibility of wild boar for *Staphylococcus aureus*, since 10 wild boars were colonized with MSSA. Beside of a multitude of differences in the living conditions of domestic pigs and wild boars, three main reasons for the difference in the occurrence of MRSA-colonization need to be discussed: 1) the antibiotic usage; 2) the animal density; and 3) the contact to animals with potential MRSA-colonization. The high frequency of MRSA carrier in the group of occupationally exposed persons to pigs compared to the frequency of MRSA in the group of non-exposed persons to livestock (<1%) shows clearly a zoonotic component in the laMRSA complex.

Conclusions

More detailed and targeted research into the risk factors of MRSA in pigs is necessary. Persons occupationally exposed to pigs should be declared as new risk group that potentially introduces MRSA into hospitals. Further research on the occurrence and distribution of MRSA ST398 in livestock and people is highly recommended as a contribution to a proactive risk management in contrast to a crisis management after MRSA ST398 has become a major contributor to the haMRSA and the caMRSA complexes.

RISK ANALYSIS OF PORCINE ZONOSSES

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Introduction

Pigs harbour several agents that have – or might have – a zoonotic impact. Transfer of antibiotic resistant zoonotic bacteria is one of such issues which have attracted attention lately. The implication of human exposure might have serious implications, and therefore, risk managers like veterinary authorities are eager to implement risk-mitigating initiatives to lower the perceived risk. However, if this is done without a risk assessment being conducted, the risk might not be addressed appropriately.

In 2006, macrolides were withdrawn from the list of antibiotics recommended for veterinary treatment of diarrhoea in Danish pigs. The motive was to lower the antibiotic consumption in general and to mitigate the risk related to human infection with macrolide-resistant (Mres) *Campylobacter*. However, a risk assessment was not conducted prior to the implementation of the risk management option.

Risk assessment is a powerful tool used to address unwanted events like spreading of zoonoses. It provides a good overview of the pathway(s) leading to the unwanted event, e.g. how people are or can be exposed to antibiotic-resistant bacteria. Moreover, it assists in identifying where the risk is non-negligible, why, and what can be done about it. Furthermore, it demonstrates where important data are missing, and hence identifies where additional scientific work is required.

Objectives

We decided to conduct a risk assessment following international guidelines to address the risk for human health associated with usage of macrolides in Danish pigs.

Materials and methods

A risk analysis consists of three elements: Risk assessment, Risk communication and Risk management. In the following, only risk assessment is described in more details. First, hazard identification is made implying that the unwanted event (presence of a specific pathogen) is identified. In the release assessment, the probability of the pathogen in live animals is assessed. In the exposure assessment the probability of people being exposed to the pathogen is assessed through the food-borne route. In the consequence assessment, the consequences related to this exposure are evaluated. That is related to how ill a person becomes when infected with a resistant version of the pathogen compared to a non-resistant. The materials used consisted of output from specific studies, official statistics, literature, report from official laboratories and expert opinion.

Results and discussion

The hazard identification revealed that development of macrolide-resistant (Mres) *Campylobacter* is the hazard of interest related to use of macrolides in pigs. Data from different EU countries show that beef contains a very low prevalence (typically 0.1-1.1%) of *Campylobacter*; moreover, Mres is uncommon in *Campylobacter* isolates from cattle (between 0 and 6%). Beef was therefore left out of further analysis. For pork at retail, a high variation in the prevalence of *Campylobacter* has been reported within EU; but generally the prevalence is <10%, and the isolates are often Mres. Danish pork harbours an even lower prevalence; (0.01%) because of use of blast chilling after slaughter. EU data indicate that poultry meat harbor a high prevalence of *Campylobacter* (>>10%) with Mres at a prevalence ranging from 0 to 8%. According to the exposure model -- that included origin of meat as well as consumption patterns -- most human cases of Mres campylobacteriosis in Denmark (157 out of 186) were ascribed to imported meat. Only seven cases could be explained by veterinary usage of macrolides in Danish pigs. In general, human cases of campylobacteriosis are self-limiting, and it is questionable whether there is any excess risk related to human infection with Mres *Campylobacter* compared to sensitive *Campylobacter*.

Conclusion

Based on the risk assessment it was concluded that the risk associated with veterinary use of macrolides in Danish pigs for the human health of Danes is low.

HEPATITIS E VIRUS (HEV) INFECTION IN PIGS: AN EMERGING ZONOTIC DISEASE?

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Introduction

Hepatitis E virus (HEV) is the causative agent of hepatitis E (HE), an important human disease in many underdeveloped countries in Asia and Africa, where it can cause up to 25% mortality in pregnant woman. HEV is mainly transmitted by faecal oral route and causes important epidemic outbreaks associated to deficient sanitary conditions in those countries. On the other hand, in industrialized countries the disease is sporadic and usually linked to travel to endemic countries. However, several studies performed in industrialized countries (including some from Europe) have been reporting HEV infections in people without history of travelling to endemic countries, suggesting the existence of autochthonous strains and/or a possible animal reservoir for the virus.

Accumulating evidences indicate that hepatitis E is a zoonotic disease. Serologic studies have shown that many species are positive to anti-HEV antibodies, including pig, wild boar, rodents, dog, cat, sheep, cow, goat, horse, rabbit, monkey and deer. In 1997, an animal HEV strain was detected and described in swine from USA and was designed porcine HEV. Such porcine HEV was highly similar to human HEV US strains previously described. Later, HEV was detected in pigs from other countries including United Kingdom, The Netherlands, Taiwan, Japan, Canada and Spain. In those countries, porcine and human HEV strains are genetically related between them. In addition, high prevalence to anti-HEV in pig population has been reported in industrialized countries, which suggests an enzootic nature of the infection in this species. Interestingly, some studies have shown that swine workers have a higher prevalence to anti-HEV antibodies compared to other professionals. Those evidences suggest an inter-species infection and that swine may play a role in the zoonotic transmission of the virus. Apart from domestic pigs, some wild animals can also act as potential HEV reservoirs. In Japan, HEV infection was reported in humans after ingestion of undercooked wild boar and deer meat. In one of these cases, HEV sequences from deer and the four patients who consumed raw meat were shown to display a 99.7-100% nucleotide identity.

Based on accumulated evidence, HEV infection seems to be ubiquitous in the swine and wild boar populations worldwide and may play a role as a potential zoonotic reservoir. Therefore, it is important for swine veterinarians to be aware on the current knowledge on HEV infection in the pig. Accordingly, the objective of this presentation will be to summarise some of the features of this swine infection.

Epidemiology

HEV infection generally takes place at 2-3 months of age, after maternal antibodies waning. The pattern of seroconversion for HEV is similar to that of typical antibody dynamics to other swine viruses. Seroconversion to IgG is mainly observed between 8 and 15 weeks of age preceded by HEV-specific IgM and IgA. In all performed studies to date, once pigs seroconvert to HEV IgG, those antibodies remain until slaughter age. IgM duration varies between 5-7 weeks and is usually related with viraemia. Adult pigs, sows and boars are usually positive for anti-HEV IgG but free from viremia and viral shedding. In one study, HEV RNA was still detected in liver and faeces of 18 week-old animals as well as in serum of 22 week-old pigs. Considering that the animals are usually sent to slaughterhouse at 22-25 weeks of age in a number of countries, it cannot be ruled out that positive animals to HEV RNA are sacrificed and, consequently, infected meat and organs may reach groceries. In fact, swine HEV RNA has been detected in 2 and 11% of raw pig liver in Japanese and American grocery stores, respectively, being even infectious for pigs. Besides, HEV RNA has been detected on muscle samples of infected animals.

Like in humans, HEV in pigs is mainly transmitted by faecal-oral route. Once an animal is infected, virus can be detected in faeces after 2-3 weeks and virus shedding can last up to 7 weeks. As soon as animals start to shed virus in faeces, it is quickly disseminated among pigs. Animals shedding virus in faeces usually have HEV in different tissues such as liver and mesenteric lymph nodes. Nevertheless, it is evident that Veterinary Diagnostic Services, probably worldwide, are receiving pigs infected with HEV for diagnostic purposes. Therefore, veterinarians and producers are probably highly exposed to swine HEV and should be aware of the potential public health concern for zoonosis of swine HEV.

Swine HEV sequences are very heterogenic and belong to genotypes 3 or 4; however, like in humans, only one serotype is assumed. Swine HEV genotype 3 has been identified in USA, Canada, Australia and many other industrialized countries from Asia and Europe. Swine HEV genotype 4 has been mainly identified in human HE endemic regions such as India and other Asian countries including Japan and Taiwan.

Clinical signs and lesions

Pigs naturally and experimentally infected with swine HEV are apparently asymptomatic. Therefore, mortality attributed to HEV infection is unknown, but probably negligible. However, HEV is able to cause hepatitis based on the analysed animals, and this lesion is usually related to virus detection in non-hepatic samples. Contrary to HEV infection in humans, natural swine HEV infection causes mild to moderate hepatitis, in coincidence with the maximum detection of virus in bile, liver, mesenteric lymph nodes and faeces.

Pathogenesis

The pathogenesis of swine HEV is virtually unknown, but it is thought to be very similar to that from humans. Major difference would be that the disease course in swine is without clinical signs. Experimental infections using naïve pigs showed that uninfected pigs housed in the same room with swine HEV inoculated animals can become infected about 2-4 weeks after the experimentally inoculated pig got infection. The primary site of swine HEV replication is not known, although virus replication in liver has been demonstrated. After replication in liver, HEV is released in gallbladder and then excreted in faeces. Urine excretion has also been described.

Using the RT-PCR assay, it has been demonstrated the existence of extrahepatic sites of swine HEV replication in small intestines, colon, and lymph nodes. Using in situ hybridization, swine HEV has been also detected in hepatocytes and bile ducts as well as in small and large intestines, lymph nodes, tonsils, spleens and kidneys.

Importantly, pigs experimentally infected with human and swine HEV strains from USA showed that both strains induce sub-clinical hepatitis in pigs. Macroscopic lesions included enlarged mesenteric lymph nodes and the main microscopic lesion was a mild hepatitis.

Zoonotic transmission

The first evidence of potential HEV inter-species transmission was drawn from experimental studies in which pigs were infected with human HEV strains and non-human primates can be infected as well with swine HEV. Second indirect evidence is the fact that humans at risk, such as swine veterinarians, are significantly more prevalent to HEV antibodies than control subjects and blood donors. Finally, high similarity between swine and human HEV strains from the same region also points out a potential inter-species transmission. As an example, a case of HE has been recently reported in a slaughterhouse worker in Spain; the causal strain showed up to 97% of nucleotide identity with swine HEV. The most convincing evidence of zoonotic transmission, however, has been reported in Japan. HE cases in humans occurred after eating raw deer meat and raw wild boar liver. In the latest cases, nucleotide identity between HEV strains detected in wild boars and patients was 99.9%.

The high prevalence of swine HEV infection together with the relatively low prevalence of seropositive swine veterinarians indicate that the likelihood of zoonotic transmission is probably low. However, such possibility should not be neglected.

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PATHOLOGY OF ENTERITIS IN PIGS

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Introduction

Intestinal infections are common diseases among growing/finishing pigs throughout the world causing substantial economic losses and decreased animal welfare. Since 1995 intestinal pathogens has been studied intensively at the National Veterinary Institute, Technical University of Denmark, with special regards to diagnosis, pathogenesis and epidemiology of *Lawsonia intracellularis*, and *Brachyspira* spp. Recently Porcine Circovirus type 2 (PCV2) and *Fusobacterium necrophorum* have been shown as noteworthy/emergent differential diagnoses in cases of necrotizing enterocolitis. This presentation provides a short review of the main activities carried out until now with special reference to in situ diagnosis and pathology.

Methods for in situ demonstration of intestinal pathogens

Specific diagnostic methods for in situ detection of *L. intracellularis* in tissue includes immunohistochemistry (IHC) using a monoclonal antibody, Law1-DK, and application of oligonucleotide probes targeting 16S ribosomal RNA of the bacterium for fluorescent *in situ* hybridisation (FISH) (1, 2). Due to strong cross-reactions between the different species of *Brachyspira*, development of IHC methods for specific detection is difficult.

Thus, for a comprehensive differentiation of porcine *Brachyspira* spp. we have developed *in situ* hybridisation methods for the specific detection of genus *Brachyspira*, *B. hyodysenteriae*, *B. pilosicoli*, *B. intermedia*, *B. innocens* and *B. murdochii* (3, 5). In contrast to immunological methods that rely on the expression of specific markers that may not be constant, phenotypic variation does not pose a problem when rRNA is used as a target.

The sensitivity of FISH is correlated to the amount of rRNA in the target organisms thus; it is strongly influenced by the physiological history and current physiological state of the bacteria e.g. starvation can result in complete lack of detectable hybridisation. Concerning *Brachyspira* spp and the use of FISH as a routine diagnostic test we recommend the colon tissue samples to be fixed in formalin as soon as possible (within half an hour) after the pig has been sacrificed. By this procedure we are able to get a high hybridisation signal as well as a well-preserved intestinal morphology for studying of the spatial distribution of the spirochetes. The effects of post-mortem autolysis of the tissue samples on the results of FISH in comparison to IHC have only been studied for *L. intracellularis*: IHC was much less susceptible than FISH to the effects of autolysis. Thus, three of nine ileum samples were FISH-negative after being kept at 20°C for 4 days, and seven were FISH-negative after 2 weeks; after 4 weeks at this temperature, however, six of the nine samples were still IHC-positive. After being kept at 4°C for 12 weeks, the majority of samples (>66%) were positive by both methods (4).

Differential diagnoses of enteritis in slaughter pigs

The applicability of *in situ* detection methods (FISH and IHC) for the demonstration of intestinal pathogens (*L. intracellularis*, *Brachyspira* spp, and PCV2) was investigated in formalin-fixed, paraffin-embedded tissue samples of the intestines (5). The pigs were submitted for routine laboratory examination with suspicion of spirochaete-associated diarrhoea/colitis. All together, 113 out of 140 pigs were positive for at least one agent, 28 pigs revealed double infections and two pigs were concomitant infected by 3 pathogens (*L. intracellularis*, *B. hyodysenteriae* and *B. pilosicoli*). The most prevalent pathogen was *L. intracellularis* (49 positive). PCV2 associated enterocolitis was detected in 23 pigs – all histopathologically characterized by varying histiocytic infiltration (macrophages and mononuclear cells) and no proliferation of immature enterocytes as seen in proliferative enteropathy caused by *L. intracellularis*. The number of pigs positive for *B. hyodysenteriae* and *B. pilosicoli* was 37 and 13, respectively. *B. intermedia* associated colitis was demonstrated in 3 cases – all associated with catarrhal colitis including invasion of lamina propria. Colonisation of colon by *B. innocens* was demonstrated in 11 cases including 2 cases of mono-infection with catarrhal colitis and invasion of crypt and surface epithelium. *B. murdochii* was demonstrated in 6 cases including 2 cases of mono-infection with catarrhal colitis and invasion of crypt and surface epithelium. Compared to *B. hyodysenteriae* and *B. pilosicoli* infections (6, 7, 10) the histopathological changes associated with the other *Brachyspira* spp. were less severe, especially in respect to surface and crypt lesions.

The importance of *B. intermedia*, *B. innocens* and *B. murdochii* as intestinal pathogens has not yet been investigated thoroughly and are by many believed to be non-pathogenic. Recently, an isolate of *B. murdochii* obtained from one of the above field cases was used in an experimental study in which 8 weaned pigs were challenged reproducing catarrhal colitis in 2 animals (8). Applying FISH, *B. murdochii* organisms were found in high numbers and closely associated with the surface epithelium, only in the pigs with catarrhal colitis. The results indicate that *B. murdochii* should be regarded as low pathogenic for pigs if present in high numbers only. Similarly *B. intermedia* and *B. innocens* may be low pathogenic if present in high numbers closely associated with the colonic mucosa. However, the diagnostic importance of culturing the bacteria from feces only is uncertain, as the method is not quantitative. Thus, development of quantitative methods such as real time PCR could be useful in future studies.

The differential diagnostic importance of PCV2 associated enterocolitis was studied further in intestines from 80 pigs submitted for routine diagnostic examination with a clinical history of *L. intracellularis* associated diarrhoea (11). Histopathologically, enteritis of varying intensity was diagnosed in 64 of the pigs. Concomitant PCV2 infection was detected in 6 out of 34 (18%) intestines with *L. intracellularis* enteritis. In the 30 other cases of enteritis PCV2 infection alone was demonstrated as the etiologic diagnosis in 23 (77%). The PCV2 associated enteritis included necrotizing ileitis and colitis grossly indistinguishable from proliferative enteritis. There was no association between presence of PCV2 and other intestinal bacterial pathogens. The result demonstrates PCV2 enteritis as an important differential diagnosis to *L. intracellularis* infection in pigs aged 2 to 4 months old with clinical history of diarrhoea.

Fusobacterium necrophorum – an emerging pathogen?

The anaerobic gram-negative bacterium *Fusobacterium necrophorum* is a long slender rod. The bacterium is considered as a part of the normal flora of the digestive system occasionally causing necrotizing stomatitis and hepatitis, however, the bacterium has recently been demonstrated in outbreaks of necrotizing enterocolitis in weaned pigs grossly indistinguishable from proliferative enteritis. In the following the first case is described (9): Two pigs were submitted for routine diagnostic investigation with a history of sudden onset of diarrhea and increased deaths within one week after weaning. At necropsy severe, acute necrotizing enterocolitis was found in both animals while the other organs appeared normal. Routine bacteriological culturing from the intestines was negative for enteropathogenic *E. coli*, *Salmonella enterica* and *Brachyspira* spp. Formalin-fixed samples of ileum and colon were immunohistochemically examined for *L. intracellularis* and PCV2 with negative result. Histopathologically, the enterocolitis was characterized by acute, deep coagulation necrosis of the mucosa with a clear demarcation zone to the vital part of the mucosa. The necrotic tissue was found severely infiltrated by long slender rods. Thus, intuitively we examined the intestinal tissue samples for *F. necrophorum* by FISH. Applying a species-specific oligonucleotide probe targeting 16S rRNA the long rods infiltrating the ileal as well as colonic mucosa of both animals were identified as *F. necrophorum*. Within the last year the bacterium has been demonstrated in another 3 herds in cases of necrotizing enterocolitis otherwise negative for intestinal pathogens. The result indicates that *F. necrophorum* in some cases should be a primary intestinal pathogen, and that it is easily demonstrated by FISH in formal-fixed tissue samples.

In conclusion, application of IHC and FISH using oligonucleotide probes targeting ribosomal RNA for detection and identification of intestinal pathogens in their natural environment is recommend, especially concerning opportunistic and low pathogenic organisms as a reliable technique for both research and routine purpose.

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BRACHYSPIRA INFECTIONS IN PIGS

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Introduction

Swine Dysentery (SD) was first described in 1921 in Indiana, USA by Withing et al 1921 (1) as a bloody diarrhoea of the large intestine. In 1971, Taylor and Alexander (2) presented evidence that a strongly hemolytic large spirochaete was the etiological agent of SD and in 1980, Taylor et al. (3) were able to produce diarrhoea in pigs by feeding them a weakly hemolytic spirochaete. The condition was described as non-fatal wasting diarrhoeal disease, designated as spirochaetal diarrhoea, today porcine colonic spirochaetosis (PCS). The present designations of the ethological agents of SD and PCS are *Brachyspira (B.) hyodysenteriae* and *B. pilosicoli*, respectively. Three other recognized *Brachyspira* spp. are frequently isolated from pigs, namely *B. innocens*, *B. murdochii* and *B. intermedia*, of which the first two are considered as non pathogenic, while the pathogenicity of *B. intermedia* remains controversial. In addition, "*B. suanatina*," isolated from pigs and mallards, has been proposed as a new species causing dysentery in pigs (4).

Present clinical situation

SD and PCS remain important endemic diseases in most parts of the world and SD is even characterized as an emerging disease, at least in parts of Europe. Antibacterial resistance in *B. hyodysenteriae* to pleuromutilins has been reported from several countries, e.g. Czech Republic, Spain and Italy.

Laboratory diagnostics

Laboratory diagnostics of SD and PCS usually rely on culture/biochemical tests and/or PCR-based methods. The PCR systems are generally based on 16 and 23S rRNA-, *nox*- or *thyA*-genes. Efficient serological assays are not available.

Present Research

The genome sequence of *B. hyodysenteriae* was recently published (5) and strong efforts are currently made to find sequences coding for proteins which could be used for vaccine development and improved diagnostics, and identification of proteins coding for virulence factors (6). A Multi Locus Sequence Typing (MLST) system for *Brachyspira* spp. has been developed (7) and is now used for characterization and studies of epidemiological relationships of brachyspiras, e.g. *B. intermedia*. Research on *B. pilosicoli* and *B. intermedia* is to a high extent pending on ongoing genome sequencing projects. Results recently presented, emphasize the importance of vectors like rodents and cockroaches in the epidemiology of SD and PCS in pig herds.

Control of SD, the Swedish experience

In Sweden a ban on antibacterial growth promoters was introduced already in 1985. During the following years, up to 1991, a continuous increase in the use of drugs with main indication SD was registered. Then the situation stabilized, and a slowly developing decrease in the use of those drugs occurred. This decrease coincided in time with a general introduction of all in–all out procedures and closure of many small production units, and an average increase in the size of the remaining herds. In 2001 a compulsory SD certification program in nucleolus- and guilt-producing herds was introduced. During 2002–2003 active tracing of SD was intensified and many eradication programs were performed in infected herds. In 2007 the estimated infection rate of SD in Sweden was very low (~1,2% of the herds).

Conclusion

Substantial achievements in the national control of SD are possible by introduction of new management practices, eradication programs performed in single herds and the availability of SD-free stock for recruitment.

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DIAGNOSIS OF ENTERITIS IN WEANERS AND GROWERS

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Objective

Determine with-in room prevalence of intestinal gross lesions during an outbreak of endemic diarrhea in a nursery room.

Materials and methods

A cross sectional pilot study was conducted in 11 herds. The herds were selected by 2 dimensional multistage sampling. Inclusion criteria for the herds were therapeutic use of in feed or in water medication for diarrhea at the room level in pigs between 10 and 70 days post weaning. Only modern intensive herds were selected. The herds were visited when there was an outbreak of diarrhea in a nursery room, where treatment by in feed or in water medication at the room level was necessary in the opinion of the farmer or herd veterinarian. Among all pigs in the nursery room 80 pigs were selected by systematic random sampling. The selected pigs were subjected to a clinical examination and a fecal sample was obtained. One observer performed all clinical examinations. A list of random numbers was used to select 8 pigs with and 8 pigs without diarrhea for euthanasia among those pigs subjected to the clinical examination. Necropsy and processing of obtained samples were performed at the National Veterinary Institute. The two pathologists performing the necropsy and all laboratory personnel were not aware of the pigs' clinical signs. Fecal dry matter content (DM%) was determined for each fecal sample using microwaves (Pedersen *et al.*, 2009). DM% was used to categorize the pigs as diarrheic or not for the statistical analyses. DM% > 18.8% was considered normal.

Results

A total of 880 pigs have been clinically examined with 38% of the pigs having diarrhea (range between herds: 25 – 48%). Eight (73%) of the investigated diarrhea outbreaks were in pigs between 12 and 35 days post weaning. Selected results of the necropsies are presented in table 1. Proliferative

DM% <18.8%	+(n = 96)	-(n = 79)
Hyperemia of small intestine	35%	32%
Proliferative enteropathy	12%	1%
Lesions in intestinal serosa, mucosa, associated lymph nodes or mesentery	49%	41%

enteropathy (PE) was detected in 4 herds. Of the PE cases 92% were in pigs more than 35 days post weaning.

The remaining intestinal lesions were dominated by hyperemia of the small intestine and occurred in pigs before and after 35 days post weaning. In contrast to PE, hyperemia occurred at similar prevalence in pigs with or without diarrhea. Prevalence of major intestinal gross lesions was investigated for different subgroups of pigs (Table 1). Large (compared to pen mates) thrifty pigs with or without diarrhea had prevalence of major lesions of 46% and 39% respectively. Small (compared to pen mates) unthrifty pigs with diarrhea had prevalence for major lesions of 75% compared to 25% for similar pigs without diarrhea.

Discussion

The current results are preliminary and no statistical analyses have been applied. This implies that the results should be interpreted with caution. The results suggest that some intestinal gross lesions occur with the same prevalence among pigs with or without diarrhea during an outbreak of endemic diarrhea. This has implications for both treatment strategies and selection of pigs for diagnostic workup. Selecting a small unthrifty pig with diarrhea will give the highest probability of selecting a pig with a major intestinal gross lesion. However, selection of a similar pig without diarrhea will give a lower probability than selecting a random pig in the room. This implies that samples for laboratory examination should be selected among pigs with both diarrhea and unthriftiness.

Conclusion

Hyperemia of the small intestine was the most prevalent intestinal gross lesion. This lesion was equally prevalent in pigs with or without diarrhea. In contrast, gross PE lesions appeared to be most prevalent in older pigs with diarrhea.

Major gross lesions were most prevalent in small (compared to pen mates) unthrifty pigs with diarrhea.

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KETOPROFEN IS EFFECTIVE IN TREATING NON-INFECTIOUS LAMENESS IN SOWS AND GILTS

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Objective

The objective of this study was to assess the efficacy of oral ketoprofen in the treatment of lameness caused by non-infectious musculoskeletal disorders in pigs.

Materials and methods

This randomized, double blinded, placebo controlled, clinical field trial was conducted in private loose-housed sows and gilts. Lactating, pregnant (≥ 100 days) and medicated animals were excluded. Altogether 1955 animals on 11 farms were walked on a hard, solid floor for at least 10 m. Lameness was scored from 0 (no lameness) to 4 (severe). The animals given a score of ≥ 2 (slight lameness) were examined clinically and the ones with any other concurrent disease including infectious causes of lameness were excluded from the study. Study animals with non-infectious lameness were given orally either placebo (N=48) or ketoprofen (Ketovet®, Vetcare Ltd) 2 mg/kg (N=47) or 4 mg/kg (N=46) for five days, when examined again. The treatment was defined successful if the lameness score on day 5 was 0 or 1 (minimal lameness). The results were statistically analysed with Cochran-Mantel-Haenszel test.

Results

Lameness on day 0 and parity of the animals did not differ between the groups ($p > 0.3$). The treatment of 25 (53.2%) animals in ketoprofen 2 mg/kg group, 25 (54.3%) in ketoprofen 4 mg/kg group and 10 (20.8%) in placebo group, was successful. The result was statistically different between the ketoprofen groups and the placebo group ($p = 0.01$), but non-significant between the two ketoprofen groups ($p = 0.8$). The treatments caused no adverse effects.

Discussion

Oral ketoprofen was efficient in alleviating symptoms of non-infectious lameness in sows and gilts. Similar findings were reported about injectable meloxicam in treating non-infectious locomotor disorders in pigs (Friton et al., 2003). In the present study, there was no difference in efficacy between the two ketoprofen dosages. The smaller dosage is both cheaper, easier to administer and has lower risk for unfavorable side-effects. As lameness is a very common problem in sows and will often lead to unplanned culling or killing of animals (Engblom et al. 2008), there is a constant need of practical ways to alleviate signs of pain and improve the welfare of sows with locomotor problems.

Conclusion

Non-infectious lameness of sows and gilts can be treated efficiently with oral ketoprofen.

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COMPARISON OF MORTALITY (ANIMAL WITHDRAWAL) RATES IN MALE FATTENING PIGS REARED USING EITHER PHYSICAL CASTRATION OR VACCINATION WITH IMPROVAC® AS THE METHOD TO REDUCE BOAR TAIN

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Objective

In most European countries, male piglets are physically castrated during the first week of life, primarily to reduce boar taint. The procedure is traumatic and leaves an open wound, creating opportunities for bacterial infection and other complications. Improvac is a vaccine alternative to physical castration that works by generating an immune response to endogenous gonadotrophin releasing factor (GnRF). Male piglets are left entire but are administered two doses of vaccine later in life, the second 4 to 6 weeks before slaughter. The second dose results in a temporary suppression of testicular function, which is followed by a reduction in boar taint and also results in behaviour similar to that of physical castrates. The objective of this analysis was to compare the impact of these two approaches on pig mortality at various stages of the production cycle.

Materials and Methods

A meta-analysis was conducted on data from 15 field studies, all performed in Europe and comparing physically castrated and vaccinated pigs. The studies were primarily conducted to compare efficacy of boar taint reduction or to look at the impact of the two approaches on growth performance. In all cases, piglets were selected for the studies and randomly allocated to treatment groups at the time of physical castration. Records were kept of all pigs that died or were withdrawn from the studies until the trials ended at slaughter, resulting in a database of 4540 animal records, well balanced between physically castrated and vaccinated pigs. Most of the pigs that were withdrawn were either too sick to continue or were in such poor condition that a producer would normally cull them, and so would equate to mortalities in commercial practice. To avoid introducing subjective judgment into the analysis, all withdrawals were considered to be mortalities.

Analytical approach

Data on mortalities-cum-withdrawals were analyzed for four time periods: castration to weaning, weaning to entry to the fattening unit, entry to the fattening unit to time of second Improvac vaccination, and time of second vaccination to slaughter. Results were summarized by study and overall and a meta-analysis was conducted on the overall results. PROC GLIMMIX was used to analyze the proportion of pigs in each treatment group that successfully completed each period as this procedure allows for the use of random effects. A binomial distribution and a logit link were used to force logistic regression for the model due to the yes/no response for completion of a period. Outputs from the regression were the least squared means and differences between the treatments. Model estimates were back-transformed to the estimated proportion removed for each treatment during each time period using the ilink option.

Results

Records were available for 2274 physical castrates and 2266 vaccinated pigs. Comparative mortality-cum-withdrawal rates for the four periods are shown in Table 1. The figures given are a percentage of the number of animals entering that phase. The mortality-cum-withdrawal rate pre-weaning was significantly higher in the castrate group. Differences in other periods were not significantly different.

Table 1. Least Squares Mean Percentage of Pig Mortality-cum-Withdrawals by Production Period

Period	Percentage of Pigs that Died or Withdrawn (\pm SE)		P-Value
	Physical castrates	Improvac Vaccinated	
Castration to weaning	4.95 \pm 0.77	3.42 \pm 0.56	0.002
Weaning until entry to fattening pens	2.76 \pm 0.75	2.73 \pm 0.74	0.96
Entry to fattening pens until 2nd vaccination	3.15 \pm 0.84	3.18 \pm 0.85	0.93
2nd vaccination to slaughter	2.48 \pm 0.44	2.05 \pm 0.38	0.19

Discussion and Conclusion

The design of Improvac field studies and the large number of studies undertaken provide a unique database of pigs monitored from a few days of age until slaughter. This analysis confirms anecdotal reports that physical castration increases pre-weaning mortality in male pigs.

CLINICAL PRESENTATION OF NECROTIC COLITIS AND TYPHLITIS ASSOCIATED WITH SALMONELLA TYPHIMURIUM IN PIGS IS NOT INFLUENCED BY THE CO-PRESENCE OF PORCINE CIRCOVIRUS 2

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Introduction

Porcine Circovirus 2 is implicated as the causal agent of Post-weaning Multisystemic Wasting Syndrome. However, there are many questions regarding its actual initiating role in the condition. PCV2 has been found to enhance the clinical expression of several post-weaning pathogens. This report describes a severe case of *S. typhimurium* necrotic colitis and typhlitis in commercial pigs associated with severe ill thrift and high mortality where PCV2 was concurrently recognized in tissues. The salmonellosis was brought rapidly under control by enhanced health management without the necessity of PCV2 vaccines, which are not licensed in Australia.

Materials and methods

Investigations were conducted on two commercial pig units. Both units were managed by the same parent company. The farms were situated 90 km north and 190 km south of Perth, Western Australia. Herd A farrows 55 sows a week and Herd B farrows 23 sows a week. Following a spike in post-weaning mortality associated with ill thrift in 3 to 10 week old pigs, a herd investigation was undertaken over a year. Following the initial investigation the farms were clinically examined each week for 12 weeks. Samples were submitted to the laboratory for routine diagnostic testing. A PCVAD scoring system based on presence of PCV2 by IHC, lymphoid depletion and granuloma formation was carried out. The association between the different clinical, pathogen and histological findings was analysed using a one-tail Z-test for two proportions at the 95% confidence interval.

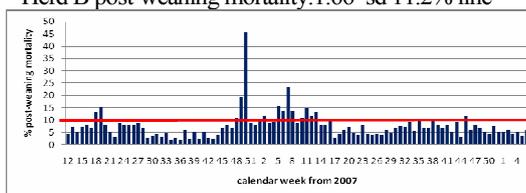
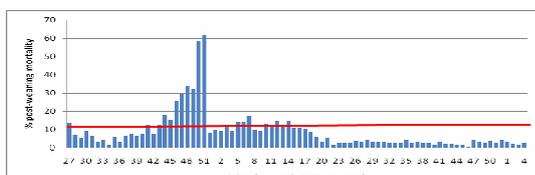
Results

Clinical signs: The major clinical sign was a yellow watery diarrhoea 10 days post-weaning in large numbers of weaners. The pure bred gilt weaners were particularly affected. Many pigs were euthanased on welfare grounds.

Mortality: Illustrated below. The mean and standard deviations are calculated prior to the outbreak week 42 A 48 B.

Herd A post-weaning mortality: 1.66*sd 11.9% line

Herd B post-weaning mortality: 1.66*sd 11.2% line



Pathology: From 110 animals post-mortemed, 72 presented with *S. typhimurium* necrotic colitis and typhlitis. While the superficial inguinal lymph node was prominent, measurements indicated no statistical difference from a sample from normal pigs of the same age. No cases of PDNS were recognised at either farm.

PCVAD score from 110 pigs (as %)			
PCVAD pig status	Herd A	Herd B	Total
Negative	64	59	63
1-3	27	17	22
4-6	5	15	10
7-9	4	7	5

Analysis of the PCV2 present revealed PCV2 type 1 at farm A and PCV2 type 2 at farm B.

Z-test correlation	Salmonella Positive	Salmonella Negative
PCVAD Positive	24	18
PCVAD Negative	49	19

The p value of 0.1459 indicates that the two variables are not correlated. Pigs with a PCVAD score and no evidence of concurrent salmonellosis often presented with an interstitial pneumonia.

Discussion and conclusion

Australia is free of PMWS, PRRSv, SIV and many other porcine pathogens. Both farms vaccinated against Parvovirus. Herd A was *Mycoplasma hyopneumoniae* negative. Both farms suffered a severe salmonellosis in the summer 2007/8. The prominent superficial inguinal lymph nodes, severe ill thrift and the isolation of PCV2 in IHC sections prompted a more detailed examination to rule out PMWS. The cause of the ill thrift was rapidly recognised as salmonellosis resulting in a classic necrotic colitis and typhlitis. Once the salmonellosis was brought under control using health management techniques, the mortality and post-weaning health returned to normal. While PCV2 was isolated and severe PCVAD lesions were recognised, in 5% of cases, this case did not satisfy the Australian definition of PMWS. Statistical analysis revealed no correlation between salmonellosis and a PCVAD score. Of the 6 animals with a severe PCVAD score, three had severe salmonellosis, one each with peritonitis, pneumonia and one with intestinal entrapment.

Occasional histological PCVAD is to be expected as PCV2 alone can create the range of histological lesions. This case suggests that PCVAD may be an incidental findings in cases of concurrent disease.

EVIDENCES OF PCV2 GENOTYPE OVERTURN IN A LONG TERM RETROSPECTIVE STUDY AND TWO LONGITUDINAL STUDIES IN SPAIN, AND THEIR RELATIONSHIP WITH PMWS CASES

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Introduction

Porcine circovirus type 2 (PCV2) is considered the essential infectious agent of postweaning multisystemic wasting syndrome (PMWS), which causes significant economic losses to the swine industry worldwide. However, PCV2 infection is not synonym of PMWS. Actually, in the field, the vast majority of PCV2 infections are sub-clinical, and only a small proportion of PCV2 infected pigs develops the clinical form of disease. Thus, even recognized as necessary for PMWS appearance, the fact that PCV2 can be present in both diseased and healthy pigs and herds indicates the need for additional associated causes or specific circumstances for PMWS triggering. One of the most recently invoked causes are virus-dependent factors, associated with differences in PCV2 virulence and viral load. Nowadays three different phylogenetic genotypes have been recognised for PCV2, and a unified nomenclature - PCV2a, PCV2b and PCV2c - has been recently proposed. In the light of such scenario, the main objective of this study was to follow up the evolution of PCV2 genotype composition during a long-term retrospective (24 years) and two farm longitudinal (14 and 7 years) studies in Spain, to seek further clarification of the PCV2 genotype infection dynamics and its relationship with PMWS appearance.

Material and methods

The retrospective study included pig sera samples used to assess the evolution of PCV2 genotypes from 1985 to 2008 in Spain. Also, pig sera samples were collected in two farms over periods of 14 and 7 years, respectively, covering before, during and after a PMWS outbreak in each farm. PCR reactions using specific primers amplified a 656 bp fragment located between positions 1284 (PCV2 ORF1 Rep) and 168 (PCV2 ORF2 Cap) of the PCV2 genome, which was subsequently sequenced and, accordingly, classified within the corresponding genotype.

Results and discussion

Historical histograms of the retrospective study and the two farms reported a swift and significant genotype shift from PCV2a to PCV2b, together with the loss of genetic variability. No PCV2c sequences were reported. The genotype overturn was related with the disease appearance in the analyzed farms, and the period of maximum PMWS epizootics in Spain in the retrospective study. All performed studies showed that the frequency of PCV2a did not increase again, after the disease epizootics. Moreover, the amount of genetic diversity reported after PMWS outbreaks was clearly lower than that detected before in all cases. The loss of diversity associated with PMWS epizootics was associated with the genotype overturn and the rise of PCV2b. Both, at general and particular levels, these studies relate the emergence of PMWS with the rise of PCV2b frequency in Spain; being the first specific report on PCV2 genotype shift associated to PMWS appearance at individual farm level. Although reasons for the association between the appearance of PMWS and the rise of PCV2b frequency are unclear, one possibility is that the PCV2b genotype possesses greater virulence or, perhaps, that PCV2b virus can escape existing herd immunity stimulated by previously circulating PCV2a strains. Present data support the hypothesis that a significant factor leading to PMWS development may be the higher virulence of PCV2b strains.

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NO CLASSICAL PMWS BUT STILL PCV2 INFLUENCE - VACCINATION AGAINST PCV2 STRAIGHTEN UP PRODUCTION

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Objective

The first cases of PMWS were observed in Sweden in 2002, when no PCV2 vaccines were available. Today, most Swedish piglet producing herds are declared free of classical PMWS. However the questions are:

- 1) Is PCV2 virus still present in these herds?
- 2) Does the virus have a negative influence on the production, even without signs of PMWS?

The objective of this study was to examine the effect of Ingelvac CircoFLEX[®] on the weight gain in a Swedish herd infected with PCV2.

Materials and Methods

The study was carried out in a Swedish multiplying herd declared free of PMWS one year earlier. Despite the lack of clinical signs of classical PMWS, the pigs were growing slower than expected. Sows were farrowing in batches (n=24) with 3 weeks interval, and pigs were weaned at 6 weeks of age. Gilt piglets were individually marked and thus traceable in the finishing unit. Within one farrowing batch, 12 litters (129 piglets incl. 54 gilts) were vaccinated with Ingelvac CircoFLEX[®] at three weeks of age, and 12 litters (132 piglets incl. 49 gilts) were kept as non-vaccinated controls. The gilt piglets were weighed at 6, 10 and 18 weeks of age. Statistical comparison was made with Students T-test with p=0.05 as significance level. Blood samples from 2 gilts/litter taken at 4, 8, 10 and 14 weeks of age were examined for antibodies against PCV2 virus to confirm the infection.

Results

At 10 weeks of age, only few pigs had antibodies against PCV2, whereas most pigs were seropositive at 14 weeks of age.

There was no significant difference in weight of the pigs between the vaccinated and the control litters at 6 and 10 weeks of age, but at 18 weeks of age, the mean weight of the vaccinated gilts was 4.9 kg higher than the non-vaccinated. The average daily weight gain from 10 to 18 weeks of age was significantly higher for the vaccinated gilts with an increase of 77 g per day. Furthermore, the variation among the 18 week old gilts was lower for the vaccinated compared to the non-vaccinated, as shown in figure 1

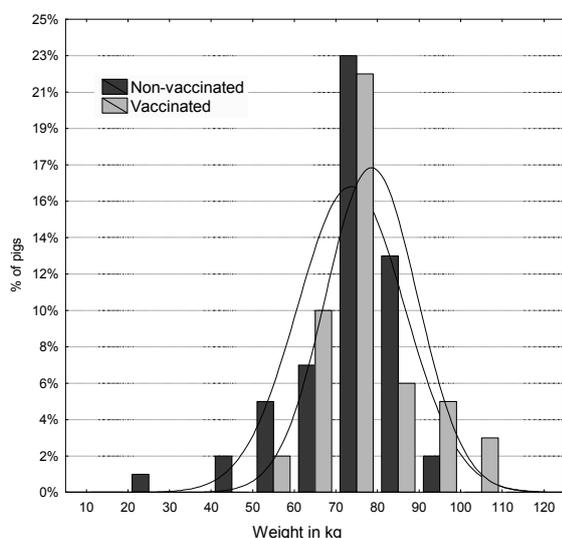


Fig. 1: Weight of gilts at 18 weeks of age.

Discussion and conclusion

The results confirm the hypothesis that a mild infection with PCV2 virus does influence the growth of pigs as seen among the non-vaccinated animals. The difference in growth occurred at the time when the infection took place. Vaccination of pigs against PCV2 results in a faster and more uniform growth of pigs as earlier shown by Fachinger, Richthofen and Kixmøller

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GASTROINTESTINAL PARASITES IN SWISS PIG FARMS – AN EXPLORATIVE OVERVIEW

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Objective

Porcine gastrointestinal (GI) parasites such as *Ascaris (A.) suum*, *Trichuris (T.) suis*, *Strongyloides ransomi*, gastrointestinal strongyles, and others occur worldwide and can significantly influence the feed efficiency, the daily weight gain, the feed intake and the health status of the individual pigs as well as the whole herd.

In most of the Swiss finishing pig farms, deworming is not routinely performed. Recent prevalence estimates for intestinal parasites are lacking. The aims of our study were thus to exploratively assess prevalence data of porcine GI parasites in Swiss pig farms and to identify potential risk factors for intestinal endoparasitosis.

Materials and Methods

The study included 110 farms: 90 conventional and 20 outdoor farms distributed all over Switzerland.

The age of the sampled pigs ranged from 4 weeks (just weaned) to ready for slaughter (about 6 months). The samples were collected in one age (weight) group on each farm. Four groups were formed as follows: weaned-40 kg (1), 41-60 kg (2), 61-80 kg (3) and > 80 kg (4). Distribution of herds was nearly equal between groups.

Rectal fecal samples were collected from pigs of the same age during the visit on the farm.

Fecal specimens were analyzed in pools of 5 samples for the presence of parasites by a combined sedimentation and flotation method using a 44% Zinc-Chloride solution. If a pool was positive, every single specimen out of that pool was individually retested. A direct quantification of the infection intensity (parasite load) was not carried out.

A questionnaire was completed during a farm visit by one person covering farm structure, animal movements, hygiene precautions, feeding, and use of drugs such as anthelmintics.

Farms were declared positive for a given parasite if one or more individual fecal samples were positive. The within group sample size was calculated using WinEpiscope 2.0. The expected threshold prevalence of gastrointestinal parasites was set at 5% and the level of confidence at 95%. The statistical analysis of the data is not yet finished.

Results

The number of samples collected ranged from 6 to 37 on outdoor housing farms and from 4 to 58 in conventional herds, depending on the number of animals of the same age on the respective farm.

In outdoor farms, *T. suis* was the most common GI parasite found, followed by *A. suum*, *Metastrongylus* sp., gastrointestinal strongyles and *Strongyloides ransomi*.

T. suis was also the most prevalent GI parasite in conventional farms, followed by *A. suum* and gastrointestinal strongyles. One farm was positive for *Strongyloides* sp. and none for *Metastrongylus* sp.

All parasites in conventional farms were found most frequently in age group (4). *T. suis* and *A. suum* showed a continuous incline of prevalence from group (1) to group (4). *T. suis* was found frequently in group (1) whereas *A. suum* was very rarely present in this group.

Discussion and conclusions

The study showed that GI parasites are frequently found in outdoor and conventional farms in Switzerland. In most cases, pigs from outdoor farms showed a higher worm burden than pigs from indoor housings. This can be explained by the higher exposition of outdoor animals to a possibly contaminated environment. Furthermore, *T. suis* seems to be the most common GI parasite in Switzerland. Its presence in conventional herds in all age groups indicates it being a problem already in breeding as well as in fattening farms. *A. suum* just plays a role in fattening herds.

Table1: GI parasites in outdoor and in conventional farms. (Percentage of infected herds. Multiple infections occurred.)

	<i>T. suis</i>	<i>Meta-Strongylus</i> sp.	<i>A. suum</i>	GI strongyles	<i>Strongyloides</i> sp.
Outdoor	60%	30%	35%	20%	5%
Convent.	32,2%	0%	13,3%	3,3%	1,1%

INGELVAC CIRCOFLEX® IS ABLE TO GENERATE A CELL-MEDIATED IMMUNE RESPONSE AFTER VACCINATION

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Introduction

Porcine circovirus type 2 (PCV2) is the causative agent of postweaning multisystemic wasting syndrome (PMWS). Traditional PMWS control has been focused on management strategies and control of concomitant diseases. However, at present, different vaccines against PCV2 have been introduced in the international market. Although all these vaccines have shown a great efficacy in ameliorating the impact of PMWS, only few studies have focused on the immunological action mechanism. Therefore, the main objective of the present work was to elucidate humoral and cellular immune responses to PCV2 in Ingelvac Circoflex® vaccinated and non-vaccinated pigs before and after challenge with a PCV2b isolate.

Materials and methods

Twenty 2-week-old conventional piglets were obtained from a commercial farm free from major pig diseases and transported to CRESA facilities. At three weeks of age, pigs from groups 1 and 2 were intramuscularly vaccinated with 1 ml of Ingelvac Circoflex® on the right side of the neck (vaccinated pigs, V) and the remaining ones (groups No. 3 and 4) were injected with 1 ml. of PBS (non-vaccinated pigs, NV). Three weeks after vaccination, pigs from groups 2 and 4 were intranasally challenged with $5 \times 10^{6.5}$ TCID₅₀/pig of a PCV2b strain (V-C [n=5], NV-C [n=7]). Pigs from groups 1 and 3 were not challenged and received PBS intranasally (V-NC [n=4], NV-NC [n=4]). Animals were clinically monitored and blood samples were collected at weekly intervals during the experiment. Pigs were euthanized and necropsied on day 42 of experiment (3 weeks post-challenge, PC). Histopathological studies included haematoxylin & eosin staining and PCV2 *in situ* hybridization. PCV2 viremia was evaluated by means of a quantitative PCR (qPCR). To evaluate the immunity induced upon PCV2 vaccination and infection, an immunoperoxidase monolayer assay (IPMA) and an IFN- γ ELISPOT assay were performed for humoral and cell-mediated responses, respectively. For the ELISPOT assay, PCV2 Capsid (Cap) and replicase (Rep) proteins were used as stimuli.

Results and discussion

At challenge, three weeks post-vaccination, no significant differences on the PCV2 antibody titres were detected between vaccinated and non-vaccinated pigs. In contrast, vaccinated pigs had developed IFN- γ -secreting cells (IFN- γ -SC) in response to the Cap protein of PCV2 by that time.

After challenge, a boost on cell-mediated responses was observed in V-C pigs. PCV2 antibody titres on day 7 PC were also significantly higher in vaccinated groups (V-C, V-NC) compared to non-vaccinated ones (NV-C, NV-VC). Significant responses against the Rep were detected only after challenge of non-vaccinated pigs, presumably as a result of a higher PCV2 replication in those animals compared to their vaccinated counterparts. Following PCV2 challenge, none of the pigs developed clinical signs compatible with PMWS during the whole experiment and no differences in the body weight evolution were detected among groups. All NV-C and 3/5 V-C pigs developed viremia. However, in the V-C group, the proportion of PCV2-positive pigs in serum decreased during the experimental period, with only one vaccinated pig being qPCR positive on day 21 PC. Pigs from NV-C group had more severe PCV2-associated lesions than those in the V-C one. Thus, from all the lymphoid tissue samples (5 tissues per pig) collected at necropsy, 22/35 from NV-C pigs had PCV2-associated lesions, while 5/25 from V-C ones. In summary, vaccination with one dose of Ingelvac Circoflex® in this experiment induced the development of humoral and PCV2 Cap-specific cell mediated immunity, reduced the proportion of viremic pigs and decreased the severity PCV2-associated lymphoid lesions.

Conclusion

One dose of Ingelvac Circoflex® induced cell-mediated immunity against PCV2.

Acknowledgements

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CURRENT SITUATION OF CLASSICAL SWINE FEVER IN BRAZIL

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Introduction:

Classical Swine Fever (CSF) or Hog Cholera is a highly contagious viral disease of domestic and wild swine. This disease is considered the major cause of economic losses to the swine industries and pig farmers because, beyond mortality and reduction of productivity, CSF leads to restrictions to the potential market and condemn the swine products. The infection can occur in diverse clinical courses depending on the virulence of the virus strain. The acute form is caused by a virulent virus strain and generally results in high mortality whereas low virulence virus could develop a chronic course. Classical Swine Fever Virus (CSFV), a *Pestivirus* from Flaviviridae family, an enveloped, positive single-strand RNA with 12kb length infectious genome, is the etiologic agent of CSF (RUMENAPT *et al.*, 1991). The advances on Brazilian CSF eradication program, CSFCEP, implemented in 1992, are evidenced by new regions declared free from the disease. Since 2001, the number of Federal States recognized as CSF-free, without vaccination regimen, increased 15 states. Although, at northeast region, considered infected area, some CSF outbreaks still occur. In this paper we analyzed the results of main CSF combat programs developed in the country from 2000 to 2009 in all Brazilian territory including CSF outbreaks in Northeast region (infected area) which was figured out by stamping out measures and compared with CSF non-vaccination areas in the same period.

Material and Methods:

The efficacy of CSF Eradication and Control Program (CSFCEP) evaluated by the outbreaks number occurred from 1992 to 2004 was studied yet in FREITAS *et al.* (2007). Data of epidemiological occurrence of CSF from 2004 until 2008 were obtained from Agriculture Defense Secretary of Agricultural Ministry of Brazil. In the CSF-Free Zone with no CSF vaccination regimen, data were plotted and analyzed by Mann-Whitney test. The survey of CSF outbreaks occurred in infected area from 2000 until 2008 was plotted and the tendency line analyzed by quadratic trend model (Minitab).

Results and Discussion:

Around the world many programs to control and eradicate CSF were applied but without plain outcome. Contaminated food, direct contact between susceptible animals with infected swine during the transport and also the use of contaminated tools are cited with the aim to explain the reasons of CSF recrudescence (TERPSTRA *et al.*, 1993). After Brazilian CSFCEP in 1992 the number of CSF outbreaks drastically reduced in all country. It should be noted that there have been no cases of classical swine fever in the Brazilian CSF-free zone since implementation in 2001. The virus, in fact, was never reintroduced, proving that the zone is secure and stimulated others states to intensify all measures to be included in CSF Free-Zone. Nowadays, the CSF Free-Zone comprises 15 states from center-west, south, southeast, north and northeast regions. These 15 States together account for almost 50 per cent of the Brazilian territory and for almost the totality of commercial swine farms. Mann Whitney analysis showed at 95% confidence level a significant difference ($p < 0.05$) between CSFCEP and oldest control program. But, in some Northeast States CSF outbreaks still occur. The comparison of profiles from CSF outbreaks data (2000-2009) plotted in the curve diagram showed an oscillatory or sinuous curve with peaks of twelve in 2001, four in 2003, and seven in 2006. Only one focus per year occurred 2007 and 2008. In 2009, until July, CSF-outbreaks reappeared in three States of North and Northeast, reaching by now 17 focuses. Those results suggest that the efficacy of implemented CSF eradication programs depends on the continuity of defined strategies as rigorous vigilance, notification, virus diagnostic screening and sanitary police measures in order to enable quick and adequate action upon CSFV detection. But, to eradicate the disease in all Brazilian territory, those measures associated with a rigorous control of animal movement in all country, backed up with serological investigations need to continue until no more outbreaks could be cited in all country.

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HAPTOGLOBIN AND C-REACTIVE PROTEIN MEASUREMENTS IN SERUM, SALIVA AND MEAT JUICES DURING PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME INFECTION

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Acute phase proteins (APPs) are useful biomarkers for distinguishing healthy animals from those suffering clinical and subclinical diseases (Petersen et al., 2001; Petersen et al., 2004; Chen et al., 2003; Pallarés et al., 2008). Haptoglobin (Hp) and C-reactive protein (CRP), considered as porcine positive fast and slow APPs, are commonly measured in serum samples in pig production (Parra et al., 2006; Pallarés et al., 2008). Recent studies have shown that other biological samples such as saliva and meat juice could be used, as alternative specimens to serum, for the measurement of APPs with several advantages. **OBJECTIVE:** The main goal of this study was to correlate the expression of haptoglobin (Hp) and C-reactive protein (CRP) in serum, saliva and meat juice of pigs experimentally infected with the European porcine reproductive and respiratory syndrome virus (PRRSV) field isolate 2982. **MATERIALS AND METHODS:** Sixteen PRRSV-free pigs were intramuscularly inoculated, killed in groups of four at 7, 14, 21 or 24 dpi, and sampled for blood, saliva and diaphragmatic muscle collection. Other four non-infected control pigs were killed at the end of the experiment. At the necropsy a macroscopic score of lung lesion was carried out and lung samples were fixed in 10 % buffered neutral formalin for the histopathological study. **RESULTS:** Significant differences in lung lesions were found between PRRSV inoculated animals and the control group, consisting on an interstitial pneumonia which still remained until the end of the study. Concentrations of Hp and CRP in serum, saliva and meat juice samples displayed a similar trend showing a peak at 21 dpi. Correlation was found for Hp and CRP concentrations between serum and saliva, serum and meat juice, and saliva and meat juice values. **DISCUSSION:** These correlations point to saliva and meat juice as alternative samples to quantify Hp and CRP levels in PRRSV infections. **CONCLUSION:** Saliva provide a minimally invasive method of sampling that could be used for monitoring PRRSV infections and meat juice is also a sample easy to obtain that could serve as alternative to serum for APPs measurements in PRRS at slaughterhouse.

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CORRELATION OF THE EXPRESSION OF IFN γ AND IFN γ -INDUCER CYTOKINES IN THE LUNG OF PRRSV-INFECTED PIGS

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IFN- γ is a cytokine with antiviral properties, which has been shown to protect macrophages *in vitro* against PRRSV replication (Bautista and Molitor, 1999). Moreover, the production of IFN- γ by pulmonary macrophages may be induced by the expression of other cytokines including IL-12, TNF- α and IFN- α (Nguyen and Benveniste, 2002; Mitchell and Kumar, 2004; Tizard, 2008). OBJECTIVE: The main goal of this study was to determine the correlation between the expression of IFN γ and those cytokines which may induce its expression in the lung of PRRSV-infected pigs. MATERIAL AND METHODS: Twenty-eight piglets were inoculated with the European PRRSV field isolate 2982, distributed in groups of four and killed at 3, 7, 10, 14, 17, 21 and 24 days post-inoculation (dpi). Other four pigs were used as control group. Lung samples were fixed in 10 % buffered neutral formalin and in Bouin solution for the histopathological and immunohistochemical studies (PRRSV, IL-12 p40, TNF α , IFN α , IFN γ antigens), respectively. RESULTS: Inoculated animals displayed an interstitial pneumonia. Increased expression of all studied cytokines was observed in inoculated animals with respect to the control animals. Cytokines immunolabelling was detected mainly in the cytoplasm of septal macrophages, and in a lesser extent in porcine alveolar macrophages and lymphocytes. Correlation was observed between the expression of IL-12 p40 and IFN γ , and TNF α and IFN γ . However, a moderate, no statistically significant, correlation was found between IFN α and IFN γ . CONCLUSION: Our results point to a main role of both IL-12 and TNF α in the expression of IFN γ in the lung of pigs infected with the European field isolate 2982. Moreover, the expression of IFN γ in our study seems to be insufficient to induce PRRSV clearance, since PRRSV was still detected at the end of the study.

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ADDITIONAL BENEFITS OF INGELVAC CIRCOFLEX® IN A CIRCOVAC® VACCINATED HERD

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Objective

Good management practices, such as the Madec rules, helped to reduce the negative effects of PCV2 infection in the herds¹, but a further significant step forward in the control of PCVD was the introduction of PCV2 vaccines. A 900-sow unit introduced sow vaccination with Circovac[®] soon after it became available in France in 2004 as its offspring experienced a severe outbreak of PCVD in 2003. Later on it added piglet vaccination with the same vaccine. Though the situation improved with the vaccination, the performance of the farm was still not satisfactory. The objective of the present study was to determine if vaccination with a new single-dose piglet vaccine, Ingelvac CircoFLEX[®] could further improve the performance and health status.

Materials and methods

The field trial took place in a 5,000 places nursery-finish unit in Brittany, France. The unit receives weekly, 21-day-old piglets from a 900-sow unit. The finishing herd is positive to PCV2, PRRS, Mycoplasma hyopneumoniae and Lawsonia intracellularis.

This side-by-side trial represented a total of 1,038 pigs. All pigs derived from Circovac[®] vaccinated sows, as sow vaccination was introduced to the unit already in 2004. Pigs were randomly assigned at weaning to two treatment groups and vaccinated either with 0.5 ml Circovac[®] (n=517) or 1 ml Ingelvac CircoFLEX[®] (n=521). Pigs of both groups were kept in separate pens, but in the same rooms. The farm staff was blinded to treatment. To determine the course of PCV2 infection serum samples were tested in qPCR at 4 time points: weaning, nursery, mid and end of finishing. Pigs were individually ear-tagged and weight at 21 days of age. The end weight was determined 140 days later before first pigs were sent to slaughter. Individual antibiotic treatments were recorded per group. Carcass quality is based on M2 and G2. M2 expresses the loin eye thickness (mm). G2 designs the backfat thickness (mm). Carcass value is calculated based on a reference price plus a bonus. The bonus is defined by the carcass quality including G2 and M2. The trial started in October 2008 and ended in May 2009.

In terms of statistics, end weights, G2, M2 and the bonus were evaluated by T-test; individual treatments and losses by Chi-square (Statistica[®] V.8, Statsoft Inc., Tulsa, USA).

Results

Mortality in the Circovac[®] vaccinated group differentiated at about 100 days post-weaning (figure 1). It was in line with PCV2 positive serum samples in mid-to-end finishing. In comparison, mortality in the Ingelvac CircoFLEX[®] vaccinated group remained at a very low level (1.0 vs. 5.6%, p<0.001). Further Ingelvac Circoflex[®] vaccinates have a significantly increased end weight and better carcass quality than the Circovac[®] vaccinated pigs (table 1).

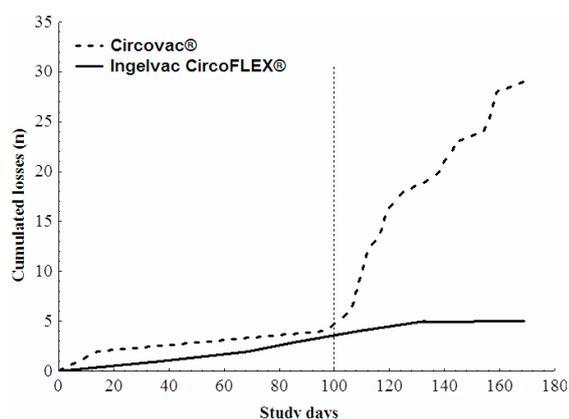


Figure 1: Evolution of losses through time

	Circovac [®]	CircoFLEX [®]	Differences	p-value
End weight (kg)	103.08 ^a	104.46 ^b	+1.38	0.04
Losses (%)	5.60 ^a	1.00 ^b	-4.60	<0.001
Individual treatment (%)	3.29 ^a	0.38 ^b	-2.79	<0.001

Carcass parameters

G2 (mm)	14.71 ^a	14.55 ^a	-0.16	0.48
M2 (mm)	59.28 ^a	60.01 ^b	+0.73	0.03
Bonus (€/kg)	0.107 ^a	0.125 ^b	+0.018	0.003

Table 1: Performance and carcass quality of the 2 vaccinated groups. (a,b: different superscripts indicate significant statistical difference)

Discussion and Conclusion

Under the conditions of this study, Ingelvac CircoFLEX[®] further improved the health status and performance compared to piglet vaccination with Circovac[®].

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IMPROVED PERFORMANCE AFTER VACCINATION AGAINST PCV2 IN A DANISH NON-PMWS HERD

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Objective

PCV2 virus is present in many Danish pig herds without causing classical PMWS. Still, PCV2-infection might affect production parameters negatively, as already shown in other countries (1, 2, 3). Therefore, Danish veterinarians often include examination of blood samples for PCV2 virus, when they work with optimization of slaughter pig production. The present study was carried out in a herd that wanted to start vaccination against PCV2 because of a moderate amount of PCV2-virus found in blood samples with qPCR. Clinical observations showed no signs of typical PMWS, and the mortality was low. Hence, a study with collection of production data was carried out. For this study, half of a batch of pigs was vaccinated against PCV2 (Ingelvac CircoFLEX[®]), whereas the other half was left as non-vaccinated controls.

Materials and Methods

The study was carried out in a Danish wean-to-finish herd producing pigs in batches of approximately 300 pigs every second week. The herd had no clinical signs of classical PMWS. Two adjacent batches of pigs were included in the study: One batch (275 pigs) was vaccinated with Ingelvac CircoFLEX[®] at 4 weeks of age, and the next batch (271 pigs) was kept as non-vaccinated controls. Vaccinates and non-vaccinates were kept in different sections. From weaning to fattening, the number of culls (pigs moved to the disease unit) and the dead pigs were recorded. When the pigs were delivered for slaughter, they were tattooed with different delivery numbers, and slaughterhouse data were collected for the two separate numbers. Statistical comparison of number of culls, deaths and lightweight carcasses was made with Chi-square test, and comparison of carcase weight and days to slaughter was made with Students T-test. Both tests used $p=0.05$ as significance level.

Group	Number of pigs		
	Culls	Dead	Lightweight ^b
CircoFLEX	9	6	12 pigs
Non-vacc.	14	4	33 pigs
Difference	+1.9%	-0.7%	-8.2%
p-value ^a	0.3696	0.7719	0.0009

^a: Yates corrected Chi-square, $p<0.05$ significant

^b: Carcase weight < 70 kg

Table 1: Number of culls, deaths and lightweight carcasses in pigs vaccinated against PCV2 compared to non-vaccinates

Results

Table 1 shows the number of culls, deaths and lightweight pigs in the 2 groups: PCV2-vaccinated and non-vaccinated pigs. Number of lightweight pigs was significantly reduced by vaccination, but number of culls and mortality was not significantly different. The number of cuts used for delivering pigs for slaughter is shown in fig. 1. The vaccinated pigs were delivered in 3 cuts, whereas 4 cuts was necessary for the non-vaccinated pigs, because >5% were not ready at the 3rd cut. 260 vaccinated and 257 non-vaccinated pigs were slaughtered, and statistical comparison showed a significant increase in carcase weight after vaccination (+2.3 kg; $p=0.0001$). The number of days to slaughter was slightly lower for vaccinated pigs, but the difference was not significant (-0.5 days; $p=0.2829$).

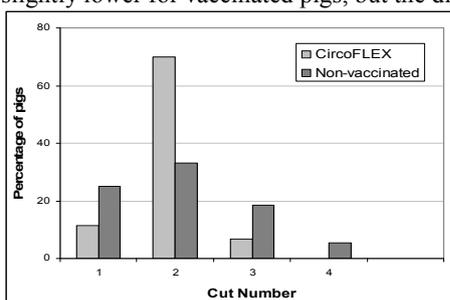


Fig. 1: Number of cuts for delivery of pigs for slaughter. 260 pigs vaccinated against PCV2, and 257 pigs kept as non-vaccinated controls

Discussion and Conclusion

The results demonstrate that vaccination of pigs against PCV2 results in production of heavier and more uniform pigs. Increased uniformity is pictured by fewer cuts and a significant reduction in number of lightweight pigs, thereby saving opportunity costs. Hence, vaccination against PCV2 improves performance, even where clinical observation of the pigs does not reveal a difference. This is probably the case in many Danish herds, considering the seroprevalence of PCV2. In herds, where qPCR shows PCV2, it is recommended to vaccinate some batches of pigs against PCV2 and collect production data to reveal the effect of vaccination

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INTRANASAL VACCINATION OF PIGS WITH A HIGHLY ATTENUATED MULTIPLE ACTINOBACILLUS PLEUROPNEUMONIAE MUTANT

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Objective

The innate immune system has an important impact onto the severity of disease after experimental *Actinobacillus pleuropneumoniae* (*A.pp.*) infection [1]. For this reason vaccine development had to account for the stimulation of the early defence mechanisms. A single aerosol application of a highly attenuated live negative marker vaccine based onto *A.pp.* serotype 2 protected significantly upon heterologous infection [2]. In order to minimize adverse effects of this vaccine on the one hand and priming the mucosal immune response on the other hand an intranasal inoculation route was tested.

Materials and Methods

Two experiments with approximately 9 week old pigs (n=12, n=14) were performed in succession. Pigs were randomly assigned to the control group receiving 0.5 ml 154 mM NaCl into both nostrils or to the vaccine group receiving 0.5 ml of the *A.pp.* mutant strain diluted in 154 mM NaCl ($4\text{-}7 \times 10^7$ colony forming units (CFU) per ml) respectively. Three weeks after immunization (p. vac.) pigs were challenged via aerosol with $1.9\text{-}5.5 \times 10^5$ CFU of a heterologous serotype 9 *A.pp.* strain per chamber (4-5 pigs). In the first trial bronchoalveolar lavage fluid (BALF), blood and tonsillar scrapings were sampled prior to, at day 4 p. vac. and at day 7 after infection (p. inf.), when pigs were necropsied. Cell counts, *A.pp.*-specific antibody titers and the Lipopolysaccharide Binding Protein (LBP) in BALF were measured. In the second trial until day 21 p. inf. only serum samples were taken to avoid any influence of invasive sampling onto the course of disease. Post mortem analysis included the bacteriological examination of palatine tonsils, bronchial lymph nodes and seven lung lobes.

Results

No difference in lung lesion scores between vaccinated and control animals could be detected. The number of organ samples positive for *A.pp.* was significantly reduced by vaccination (p=0.04) and negatively correlated to the *A.pp.*-specific antibody titers at day 21 p. inf. (p=0.005). Nine non-vaccinated but only one vaccinated pig were positive for *A.pp.* in tonsils at the end of the experiment. The vaccine strain could be reisolated in three vaccinated animals at the end of the experiment. Seroconversion took place in 8 of 13 vaccinated pigs until day 21 p. vac. and not in the control pigs. Seven days p. inf. serum antibody titers were significantly higher in vaccinated pigs. At day 4 p. vac. vaccinated pigs showed higher counts of neutrophils and macrophages in BALF and a tendency to a higher proportion of neutrophils on the cost of macrophages and lymphocytes could be observed in tonsillar scrapings. The LBP concentration was positively correlated to neutrophil and lymphocyte concentrations in BALF at day 7 p. inf. without showing any consistent changes during the course of the experiment.

Discussion

Insufficient protection in the challenge experiment might be due to the lack of the strongly cytotoxic ApxI toxin in the vaccine strain, which is produced by the challenge strain. A counter-argument is the strong impact of the innate immune responses on the severity of disease. The low isolation rate of *A.pp.* in tonsils from vaccinated pigs might be due to the presence of activated neutrophils. This unspecific response was in general reflected by changes in the cell composition in BALF and tonsillar scrapings, but not by LBP concentrations. LBP immediately binds bacterial lipopolysaccharide and potentiates the innate immune response via Toll-like receptor 4 and NF κ B [4]. Day 4 p. vac. might have been too late to detect these early mechanisms. A future approach might be the use of bioadhesive intranasal delivery systems in combination with a mucosal adjuvant to induce more potent mucosal immune reactions [3].

Conclusion

Innate as well as adaptive immune responses were triggered by intranasal vaccination with an attenuated live negative marker vaccine but were not sufficient to protect against a heterologous challenge with an *A.pp.* serotype 9 strain. The bacterial burden with *A.pp.* was significantly reduced by vaccination.

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TEMPORAL TREND IN ANTIMICROBIAL REQUIRING GASTROINTESTINAL DISEASES IN DANISH FINISHER HERDS, 2002-2007

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Objective

The objective was to evaluate the temporal trend in antimicrobial (AM) demanding gastrointestinal (GI) diseases in Danish herds with finishers on national level.

Material and methods

Information on usage of AM and herd size for the period 2002-07 was extracted from the Danish register of Veterinary Medicine and the Zoonosis Register. The study population was herds with finishers. Herd size was described by number of finishers delivered to Danish abattoirs. The development of AM-requiring GI-diseases was defined by number of herds with prescriptions and by amount of AM in Animal Daily Doses (ADD)¹ per finisher per quarter per year. Decomposition of data on trend, season and residuals using loess regression² was performed and presented graphically.

Results

The number of herds with deliveries to Danish abattoirs was reduced from 14, 086 in 2002 to 8,613 in 2007, and the number of delivered pigs was reduced by 22.6 mio. to 19.1 mio. pigs in the same period.

The proportion of finisher herds per quarter with AM-usage for GI-diseases had doubled from 0.13 in 2002 to 0.25 in 2007 (Fig. 1). An increase in ADD-usage appeared from 1.0 in 2002 1st qtr to 1.4 in 2003 2nd qtr (spring); hereafter it was relative stable. A small seasonal variation with a drop in spring was found.

Discussion

The increasing proportion of herds with AM-usage and a relatively stable AM-usage per finisher over the years can be explained by: a concentration of finishers into fewer herds with larger herd size; a development in production type from integrated to more sectioned systems as well as better bio security and hygiene, reducing the transmission and thereby the burden of disease; and a more targeted treatment-strategy due to e.g. increased supervision by veterinarians. More accurate and systematic recordings could also have influenced the results. By using deliveries to Danish abattoirs as proxy, finishers slaughtered abroad were excluded from the analyses. As an increasing percentage were exported (1.9 % in 2004 to 4.5 % in 2007³) could this have biased the results with a false higher amount of add per finisher in the more recent years.

Conclusion

From 2003 to 2007, AM-requiring GI-diseases in finishers have not increased on national level but are distributed to a larger proportion of herds. This study is an example of decomposing register data for assessing temporal trends.

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THE NUMBER OF MAMMARY GLANDS AND TEAT CANALS IN CULLED BREEDING SOWS

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Objective

The objectives of this study were to characterize the number of mammary glands and numerical evaluation of teat canals of randomly selected teat samples in slaughtered culled breeding sows in Hungary.

Materials and Methods

The investigation was carried out in front of the slaughter line just after bleeding – in 12 seconds/animal – counting the all mammary glands of the individual animals, documented by voice recorder and by making a photo depending on the position when moving. Randomly selected trimmed teat samples (0-3 samples/animal) irrespective of any pathological condition were collected separately according to their position. The number of teat canal was evaluated later by their cross sectional area. Supernumerary teats with reduces size were disregarded. The teats were numbered from 1 to 8, anterior to posterior both left and right(L1, L2, R1,R2, etc).

Results

The data of 1507 breeding sows (number of mammary glands/animals): **10/2**(0.13%); **11/89** (5.9%); **12/211**(14%); **13/352**(23.46%); **14/560**(37.16%); **15/193**(12.8%); **16/73**(4.8%); **17/27**(1.79%); **18/0** (0%); **14-17/853**(56.60%). The number of teat canals based on 901 samples collecting from mammary glands of different positions: 1canal in 130(14.43%); 2 canals in 753(83.57%) as well as 3 canals in 18 teats (2.0%).

Discussion and Conclusion

Traditionally, sows had 2.5 more teats than the number of piglets in their average litter(4). Now, the litter of 13 is not rare and the number of optimal/minimal mammary gland in these cases ought to be 15-16. This in the case of 1507 culled breeding sows (approx. 5% of total number of breeding sows in Hungry) is only 17.6%. The selection of females with more teats or more functional teats would have no positive effect on increasing litter sizes or weights at 21 d or at weaning (1,2,3). Kim et al. (5) stated that 14 or more teat number compared to 11–13 teat number in gilts increased litter size at birth(1.5-1.9)and at 21 day weaning(2.1-2.2). Most authors [4,6] claim that there are 2 canals in each teat. However, others (7) investigating 106 mammary glands found 2 canals in 90.5%, 3 ones in 7.5% and 1 teat canal in 1.9%. They estimated a practical significance of these data. Our data not detailed here does not confirm this.

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RETROSPECTIVE STUDY OF SWINE TORQUE TENO VIRUS (TTV) GENOGROUPS 1 AND 2 INFECTION IN AUSTRIA IN 2008 INCLUDING A CORRELATION BETWEEN THE OCCURRENCE OF TTV AND THE PORCINE CIRCOVIRUS 2 (PCV-2) AND CLINICAL CASES OF PDNS

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Objective

The torque teno virus (TTV) is a newly discovered virus in humans and several domestic and wild animal species including swine (Kekarainen and Segalés, 2009). The virus belongs to the genus Anellovirus. Two TTV genogroups could be identified in the domestic pig as well as in the wild boar (Niel et al., 2005; Martinez et al., 2006). The regional distribution of TTV1 worldwide is variable and ranges between 33 and 100 % (Segalés et al., 2009). However, the prevalence of TTV2 is lesser known and has been investigated in correlation to PCV-2 infections (Segalés et al., 2005) indicating a prevalence of 77 % in Spanish pig farms (Kekarainen et al., 2006).

Materials and Methods

Sera were collected from 83 farms which were located in Austria's main swine producing regions in 2008. A total of 250 sera were tested by a conventional PCR (Segalés et al., 2009). 58.5 % of the samples were taken from weaned piglets, 19.0 % from finishing pigs, 8.3 % from sows, 6.3 % from suckling piglets and 5.5 % from gilts. The ages of 2.4 % of the sampled pigs were unknown. Furthermore, samples from 51 pigs with clinical symptoms indicating PDNS were tested by in situ hybridization for the occurrence of PCV-2 genome and by conventional PCR (Segalés et al., 2009) for TTV1 and 2.

Results

A total of 80.7 % of the 83 sampled farms were positive for TTV. 42 of the farms tested positive for TTV1 as well as TTV2. 13 tested positive only for TTV1 and 12 farms only for TTV2. 16 farms were negative for both genogroups. 12.5 % of the samples from the suckling piglets were TTV positive. In addition, 59.5 % of the weaned piglets, 79.2 % of the finishing pigs, all of the tested gilts, and 33.3 % of the sows tested positive for TTV. 64.7 % of samples taken from the 51 pigs showing clinical symptoms of PDNS tested positive by in situ hybridization for PCV-2. 35.3 % of these PCV-2 positive samples were also TTV1 and 54.3 % TTV2 positive.

Discussion

Data compiled in this retrospective study indicate that both swine genogroups TTV1 and TTV2 are circulating in Austria. The detected Austrian farm prevalence of 80.7 % was comparable to the 90.5 % prevalence found by Brassard et al. (2008) in Canada. However, this prevalence was higher than data found by Martelli et al. (2006) in Italy and McKeown et al. (2004) in six different countries worldwide. In contrast to Segalés et al. (2009), who showed that TTV2 was significantly more prevalent than TTV1 in Spain, this present study did not observe any differences between TTV1 and TTV2 prevalence. This present study found that both genogroups were more prevalent in postweaning pigs. From all 51 investigated PDNS cases, only 64.7 % tested positive by in situ hybridization for PCV-2, which could be defined as the gold standard. TTV2 was more prevalent than TTV1.

Conclusion

Both TTV genogroups were circulating in Austrian farms with nearly the same prevalence in the year 2008. This study found that postweaning pigs appeared to be predominantly infected. In the clinical PDNS cases, TTV2 was more prevalent than TTV1.

Acknowledgments

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COMPARISON AND ECONOMIC EVALUATION OF TWO PCV2 VACCINATION PROGRAMS USED IN FRANCE

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Objective

First described in West Canada in 1996, PMWS was seen soon after in France¹. Nowadays the virus PCV2 is recognised as being ubiquitous and the PCV2-related diseases (PCVD) have a major impact on the world swine production. Different vaccination programs exist and help to control the disease.

The objective of this trial was to compare the efficacy and economic benefits of a new single dose PCV2 piglet vaccine (Ingelvac CircoFLEX[®], 1 ml), with the previously established piglet vaccination in the herd (Circovac[®], 0.5 ml).

Materials and methods

The trial was carried out in a 700-sow farrow-to-finish unit in Brittany, France, with a weekly production rhythm and weaning at 21 days of age. The herd was positive for PCV2, PRRS, APP, Mycoplasma hyopneumoniae and Lawsonia intracellularis. Sow vaccination with Circovac[®] was introduced in mid 2006 and shortly before the start of the trial piglet vaccination with 0.5 ml Circovac[®] was established in addition to that.

A total of 1,198 pigs were randomly assigned at weaning to two treatment groups and individually ear-tagged: 599 piglets received intra-muscularly 0.5 ml of Circovac[®], 599 piglets were injected intra-muscularly with 1 ml of Ingelvac CircoFLEX[®]. Pigs of both groups were kept in separate pens, but in the same rooms. The farm staff was blinded to treatment. To determine the course of PCV2 infection 9 blood samples were taken per group at 4 time points and tested with PCR (3 pools of 3 samples each): weaning, nursery, mid and end of finishing. Pigs were weighed individually at weaning (~21 days of age) and on average 140 days later before first pigs were sent to slaughter (end weight). Pigs having an end weight of 25% below the average of all pigs on trial are defined as culls. Individual carcass data were collected at slaughter. Live weight at slaughter was calculated back from the carcass weight and was used together with the individual slaughter age to calculate the wean-to-finish average daily gain (ADG w-f). Individual antibiotic treatments were recorded per group. Based on the individual performance and mortality data the economic benefit of vaccination was calculated as gross margin per pig (GM, in Euro €). The GM equals the revenue generated by each pig minus its piglet and feed costs. Price references²: €1.20/kg pork price, €20/piglet and €200/ton feed.

In terms of statistic, weaning weights, end weights ADG w-f and GM was evaluated by T-test; individual treatments, culls and mortality by Chi-square (Statistica[®] V.8, Statsoft Inc., Tulsa, USA).

Results

The presence of PCV2 during the trial was confirmed through PCR positive samples mid and end of finishing. At the same time the difference in mortality between Circovac[®] and Ingelvac CircoFLEX[®] became very obvious leading to a difference of wean-to-finish mortality of more than 60% (3.34 vs 8.68%, p=0.0001, table 1).

The Ingelvac CircoFLEX[®] vaccinated pigs performed significantly better than the Circovac[®] vaccinates, resulting in a significantly higher GM (table 1). In addition, Ingelvac CircoFLEX[®] vaccinated pigs were clinically healthier, as reflected in a significantly lower number of animals treated with antibiotics (3.67 vs 6.34%, p=0.034).

	<i>Circovac</i> [®]	<i>Circovax</i> [®]	<i>Differences</i>	<i>p-value</i>
Weaning weight (kg)	5.55 ^a	5.49 ^a	-0.06	0.31
End weight (kg)	86.51 ^a	88.65 ^b	+2.14	0.006
ADG w-f (g/day)	598 ^a	612 ^b	+14	0.002
Mortality (%)	8.68 ^a	3.34 ^b	-5.34	0.0001
Culls (%)	7.85 ^a	3.28 ^b	-4.57	0.0058
GM (€/pig)	20.0 ^a	27.8 ^b	+7.8	<0.0001

Table 1: Performance and gross margin of the 2 vaccinated groups. (a,b: different superscripts indicate statistical significant differences).

Discussion and Conclusion

In a PCV2 vaccinated sow herd the pigs of the Ingelvac CircoFLEX[®] vaccinated group had a lower mortality, less culls, increased weight gain and less antibiotic treatments compared to the Circovac[®] vaccinated animals. These findings indicate a superior efficacy against the negative impact of PCV2 infection, which resulted in a significant gross economic benefit of +7.8 € per pig.

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IMPLANTABLE MICROCHIP TRANSPONDERS FOR BODY TEMPERATURE MEASUREMENTS IN PIGS

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Objective

Body temperature is a simple, but clinically important parameter in monitoring the health status of pigs, both at individual level and herd level. The standard procedure for obtaining such data is normally performed by recording of the core body temperature, using a rectal digital thermometer. This work, however, can be quite time consuming and laborious, and further compromising the immediate well-fare of the pig, when restraining of the individual animal is necessary. Therefore, an electronic body monitoring system using implantable microchip transponders for measuring peripheral body temperature was tested, in order to evaluate the utility and reliability of this tool, in domestic pigs. The system is presently used and well optimized in small laboratory animals [1, 2]. We tested the microchip transponders during experimental infection of pigs with classical swine fever virus (CSFV), a viral infection, which can cause high fever in infected animals.

Materials and Methods

Implantable, programmable temperature transponders (IPTT-300TM) from Bio Medic Data System (Plexx, the Netherlands), designed for non-surgical implantation into animals, was tested in 30 weaner pigs. One microchip transponder was injected deep subcutaneously by the left ear base of each individual. The transponder was before insertion programmed with ID identical to the individual pig's ear tag number. The pigs were randomly divided into 3 groups: one group placebo-infected and two groups virus-infected with 2 different strains of CSFV, one of them known to induce pyrexia. Peripheral body temperature recorded from the transponder by a hand-held scanner and core body temperature recorded by a conventional rectal digital thermometer was registered on a daily basis for 3 weeks. The data set obtained for the 2 methods were compared. As the pigs included in this experiment were sequentially killed and hence the number of pigs within each group was reduced with 3-4 individuals per week, the corresponding data sets diminished during the experimental period.

Results

All virus inoculated pigs were infected with CSFV, as determined by virus detection. So, the transponder system was tested in both clinically healthy and clinically ill pigs with physiologically normal body temperature or fever, respectively. The data obtained in this study, showed a correlation between the two methods for monitoring body temperature. The peripheral body temperature measured by the microchip transponder was on average 0.5-1.0 °C lower than the core body temperature measured by rectal thermometer, in all 3 groups. However, the paired data sets followed the same pattern throughout the experimental period. Standard deviation of the mean values of body temperature on the individual days in the respective groups was larger for the transponder data compared to the rectal data, indicating less accuracy for the monitoring system when used on individual animal level.

Discussion and Conclusion

Comparison of the data sets from the 2 methods showed that the peripheral subcutaneous body temperature recorded by a microchip transponder may be interesting as a monitoring tool in the clinical surveillance of the health status of domestic pigs. This technology has not at present the power to monitor individually sick pigs, where accurate core body temperature is important for supervision and treatment of the specific animal. However, on group or herd basis this system could be valuable in overall herd surveillance where data on body temperature could be included as an early indicator of changes in infectious disease status.

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RAPID DETECTION OF SWINE INFLUENZA VIRUS COLLECTED IN FTA CARDS BY RT-PCR

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Introducción

We wish to report on the feasibility of using FTA[®] cards (Whatman [GE Healthcare], NJ, USA) for sample collection of clinical specimens from pigs, and subsequent detection of the swine influenza virus (SIV) using RT-PCR assays. The FTA card is a cotton-based cellulose membrane impregnated with lyophilized chemicals designed to capture and bind DNA samples for future processing. The aim of the study was to verify whether FTA cards allow the RNA from SIV subtypes H1N1, H3N2 and H1N2 to be preserved when stored at room temperature over a given period of time.

Materials y methods

Two groups of samples were tested: a) Low-passage clinical SIV isolates (n=3) A/swine/Spain/53207/2004(H1N1), A/swine/Spain/58571/2004(H3N2) and A/swine/Spain/40564/2002(H1N2) propagated in SPF embryonated chicken eggs and b) Clinical material from unrelated clinical outbreaks (n=10) of pig respiratory disease, consisting of nasal swabs kept in virus transport medium (n=4) and lung tissue homogenates (n=6). Samples came from sows and growing pigs showing clinical signs of influenza, or dying from acute pneumonia. Before FTA cards were inoculated, all samples (n=13) were confirmed positive for SIV by a type A influenza-matrix gene RT-PCR adapted to the SYBR Green real-time format in the SmartCycler[®] system (SYBR Green RT-PCR). One hundred microliters of each sample and SIV isolate were dropped directly onto the circle marked for samples on the FTA Card in a concentric circular pattern. Cards were allowed to air dry and were stored at room temperature. On day 7 post-inoculation, a triangular-shaped piece of the paper was carefully cut, transferred to a sterile Eppendorf tube and processed for total RNA extraction (RNeasy Protect Mini Kit, Qiagen, Madrid, Spain) following the RNA cleanup protocol in the kit manufacturer's instructions. The eluted RNA from the clinical samples (nasal swabs and lung homogenates) was subjected to SYBR Green RT-PCR for SIV detection; SIV isolates subtyping was achieved by four conventional, gene-specific RT-PCR assays (RT-PCR) targeting the H1, H3, N1 and N2 SIV genome segments 2.

Results

All SIV-containing FTA specimens rendered positive results in the PCR assays on day 7 post-inoculation. Furthermore, the RT-PCR signal showed no apparent reduction over time, indicating that SIV RNA in allantoic fluid and tissue homogenates remained stable on the cards, regardless of the virus subtype.

Discussion

These results demonstrate the usefulness of FTA cards for the storage and subsequent detection and typing of the SIV RNA genome by real-time and conventional RT-PCR. The current flu pandemic is caused by influenza A virus subtype H1N1 that has caused illness in individuals globally. In response to the increasing risk of reverse zoonosis occurring in densely populated pig areas¹, it is very likely that it will become necessary to increase the level of nationwide surveillance of influenza activity in pigs. The main drawback for laboratory confirmation of SIV outbreaks, even when sampling is performed in a timely manner, is the relatively labile nature of the virus, with a half-life of a few hours at room temperature. Microorganisms preserved on FTA cards become inactivated; therefore, they cannot be isolated³. However, getting an early diagnosis through the combination of sample submission on FTA cards followed by RT-PCR detection would increase the responsiveness of diagnostic laboratories. Ongoing work in our laboratory using the proposed methodology is addressed to assess the sensitivity of the whole procedure using serial dilutions of titrated SIV suspensions.

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IMPACT OF PCV2 VIREMIA IN VACCINATED AND NON-VACCINATED PIGS

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Objective

PCV2 vaccines have proven to be very effective in protecting pigs against the negative impact of PCV2 infection. One parameter that was used in initial trials to evaluate vaccines was PCV2 viremia. This was based on the fact that in numerous trials in non-vaccinated animals a paradigm had been established that pigs with a higher viral load have a higher chance to be affected by PCVAD. Furthermore, in contrast to PCV2 viremia, clinical signs and mortality cannot be reproduced consistently in lab trials after PCV2 challenge. The objective of this study was to analyse the impact of PCV2 viremia in vaccinated and non-vaccinated pigs in commercial farms and to evaluate if PCV2 viremia is a useful tool to assess vaccine efficacy in the field.

Materials and methods

The study included 4 different sites in Europe (Northern (N.) Germany, Southern (S.) Germany, France, UK) with different types of co-infections. Three of the four sites being positive for PRRS and *Mycoplasma hyopneumoniae* (1-4). Piglets were included in the study and injected with a single dose (1 ml) of Ingelvac CircoFLEX® (n=2854) or Placebo (n=2748) at about 2 to 4 weeks of age. A subset of pigs were bled every or every other week (490 vaccinates, 475 controls) and tested in qPCR for PCV2 viremia and viral load. Pigs were individually weighed at inclusion and at the end of the trial to determine average daily gain (ADG).

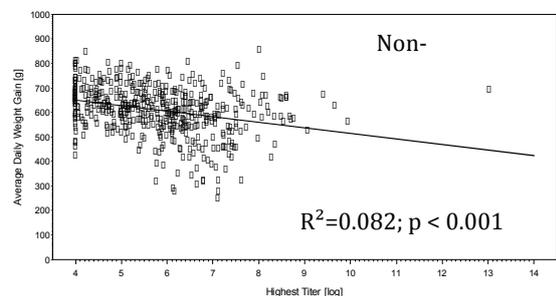
A linear regression model was applied to test for the correlation between the highest viral load (genomic equivalents/ml of serum) found in each individual animal during the trial and its ADG (SAS System, SAS Inst., Cary, North Carolina, v 8.2).

Results

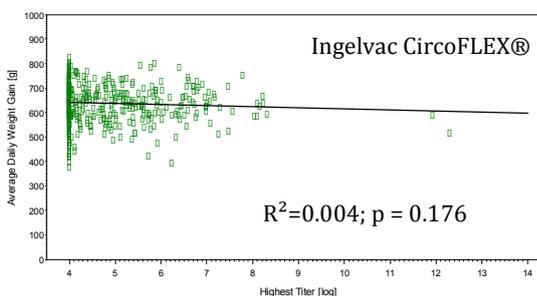
In all 4 study sites the number of viremic animals, duration of viremia and viral load were significantly reduced in vaccinated animals compared to the control group, as reported earlier (1-4). A significant, though weak, negative correlation between the highest viral load and ADG was detected in non-vaccinated animals ($p < 0.001$, figure 1a). In contrast to that, no correlation was found in the vaccinated pigs ($p = 0.176$, figure 1b).

Fig. 1: Correlation between viral load and ADG

a) Significant correlation in control group (n=475).



b) No correlation in vaccinated group (n=490).



Highest titer (x-axis) shown as logs of PCV2 genomic equivalents per ml of serum.

Discussion and conclusion

Residual viremia has been found in vaccinated animals in various studies, independent of the PCV2 vaccine used (1-5, 7). However, this study confirmed the findings of Holck et al (6) that residual PCV2 viremia is not affecting the performance of PCV2 vaccinated pigs, in contrast to the situation in non-vaccinated animals. Furthermore, this is in line with a trial that showed no relation between the number of viremic pigs and the mortality rate in groups vaccinated with different vaccines (7). PCV2 viremia might be a valuable parameter comparing vaccinated and non-vaccinated animals but is not a useful parameter when assessing efficacy among vaccinated animals only.

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EFFICACY OF INGELVAC CIRCOFLEX® IN THE PRESENCE OF HIGH MATERNAL ANTIBODIES IN FOUR EUROPEAN FIELD TRIALS

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Introduction

In recent years, PCV2 piglet vaccination has become global routine use. Since the beginning of vaccine use it has been discussed if vaccines work effectively in the presence of high maternal immunity. It has been repeatedly demonstrated that Ingelvac CircoFLEX® induces protective immunity even in the face of high maternal antibodies (1,2,3). However, with other vaccines a possible interference was described based on the serological response to vaccination (4,5), suggesting a maternally derived antibody (MDA) titre of 1:320 as a possible cut-off (5). The present paper evaluates four placebo-controlled, side-by-side studies from Europe comparing the efficacy of PCV2 piglet vaccination in pigs with MDA titres below or above 1:320.

Materials and Methods

Efficacy data was collected from four different study sites throughout Europe (Northern (N.) Germany, Southern (S.) Germany, France, UK). Piglets were included in the study and on the same day injected with a single dose (1 ml) of Ingelvac CircoFLEX® or Placebo at about 2 to 4 weeks of age. All pigs (2854 vaccinates and 2748 controls) were bled at inclusion to determine PCV2 MDA titres with an indirect fluorescence antibody titration (IFAT) assay. The method allowed the detection of antibody titres in a range from 1:5 to 1:20480. Pigs were categorized in two groups, MDA titre > 1:320 or MDA titre < 1:320. The primary efficacy parameter was weight gain. Weight gain was defined as the difference between individual weight at the end of the trial and the weight at inclusion.

Weight gain differences were analysed with a two-sided t-test (SAS System, SAS Inst., Cary, North Carolina, v 8.2).

Results

Antibody titres at vaccination ranged from 1:10 to 1:20480. In total, 3218 pigs had an MDA titre < 1:320 at inclusion (1571 controls, 1647 vaccinates) and 2375 pigs a titre > 1:320 (1177 controls, 1198 vaccinates). No significant difference was observed between vaccinated pigs that had an MDA titre > 1:320 compared to those with a titre < 1:320 (Table 1). Comparing vaccinated and control pigs with a titre > 1:320, vaccinated pigs showed a significant increase in weight gain in N Germany, S. Germany and the UK ($p < 0,0001$, Table 2). Due to a relatively low number of high titre animals in the French trial (298 controls, 292 vaccinates) the difference in weight gain in favour of the vaccinates did not reach statistical significance ($p=0,0564$).

Table 1: Weight gain (kg) in vaccinated pigs with high or low MDA titre at vaccination.

Study site	Titer < 1:320	Titer > 1:320	p
France	89,3	89,1	0,8464
N. Germany	103,3	103,1	0,8003
S. Germany	99,5	99,3	0,8473
UK	85,9	86,8	0,2997

Table 2: Weight gain (kg) in pigs with an MDA titre > 1:320 at vaccination. Controls versus CircoFLEX.

Study site	Placebo	CircoFLEX	p
France	87,1	89,1	0,0564
N. Germany	99,6	103,1	< 0,0001
S. Germany	95,0	99,3	< 0,0001
UK	81,8	86,8	< 0,0001

Discussion and Conclusions

In this evaluation maternal antibody titres >1:320 at vaccination did not have a negative impact on vaccine efficacy. This confirms the findings described in previous papers demonstrating lack of interference with maternal immunity when pigs were vaccinated with Ingelvac CircoFLEX® around weaning (1, 2, 3, 6). It can be concluded that Ingelvac CircoFLEX® effectively breaks through high levels of maternal antibodies from two weeks of age onwards.

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THE COMBINATION OF BOTANICAL ANTIOXIDANTS AND PROBIOTICS IMPROVE PRODUCTION PARAMETERS IN WEANED PIGLETS

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Objective

Weaning is a traumatic period for the suckling piglet due to changes in feed, immune status and environment, often resulting in health problems and production losses. Botanical antioxidants and probiotics have proven positive effects on health and production in weaned piglets, but a combination of botanical antioxidants and probiotics might result in an even better protection of the piglet due to different mode of actions. Extracts of olive leaves have a high content in oleuropeosides, flavonols and substituted phenols, which bring about a reduction in oxidative stress inhibition in oxidation of LDL and an increase in antioxidant cellular defence (Covas et al., 2006). Extracts of grapes are rich in anthocyanins and condensed tannins, which protect cells against oxidative stress, preserve vitamin E and nutrients in the organism and have an immune stimulating effect (Saura-Calixto, 1998). Probiotics work by restricting coliform and promote lactic acid producing bacteria. The objective of the experiment was to investigate the effects of including botanical antioxidants from grape and olive leaves and probiotics (*Enterococcus faecium*) (GOP) on selected production parameters in weaned piglets.

Material and methods

A total of 120 newly weaned piglets 28 days of age (liveweight 7.3 ± 0.9 kg), half castrates and half gilts were randomly allocated to 2 treatments (supplementation of 0 and 2000 ppm of GOP) balanced for liveweight and sex. There were 4 replications per treatment and 30 pigs per replicate. All pigs were fed with a standard feed ration based on barley, wheat, soybean and rice meal. Piglets were weighed individually and feed consumption was registered by replicate at start of the trial, after 11 days, 21, 31-32 and 42 days respectively. Data was analyzed using the GLM procedure of SAS. The trial was carried out at a commercial farm in Denmark.

Results and conclusion

Supplementing piglets with a blend of natural antioxidants and probiotics resulted in improved daily gain in the period of 42 days following weaning with 421 g/day compared to 390 g/day in the control group ($P < 0.05$) (Table 1). Feed conversion was 1.70 and 1.57 kg feed/kg gain in the control and trial group respectively. It can be concluded that the combination of botanical antioxidants and probiotics have positive effects on production parameters in piglets.

Table 1 Effect on production parameters of supplementing weaned piglets with botanical antioxidants and probiotics

	Control group		Trial group		P-value	
	Mean	STDV	Mean	STDV	Group	Gender
Av. start weight, kg	7.3	0.9	7.2	0.8	NS	NS
Av. end weight, kg	23.8	4.9	25.1	4.8	NS	NS
Average daily gain/pig, g						
Day 0-11	211	86	189	86	< 0.05	NS
Day 11-21	299	96	297	106	NS	NS
Day 21-31	437	180	497	169	< 0.0001	NS
Day 31-42	607	251	692	190	< 0.0001	NS
Total (day 0-42)	390	116	421	117	< 0.05	NS

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OBJECTIVE ASSESSMENT OF FAECAL CONSISTENCY IN PIGS PART 1: INTER-OBSERVER AGREEMENT FOR ASSESSMENT OF FAECAL CONSISTENCY

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Objective

The objective of this pilot study was to evaluate inter-observer agreement for assessment of faecal consistency in growing pigs using a simple consistency score with 3 categories.

Materials and methods

Three observers with at least five years practical pig veterinary experience working with pigs were included by convenience in the study. No calibration was allowed between observers prior or during the study.

A total of 320 faecal samples were collected in 4 Danish herds (80 samples per herd).

During the visits to each herd growing pigs (12-14 weeks old) were selected at convenience, a numbered ear tag applied and a faecal sample collected by Observer 1 using rectal palpation with a gloved hand. Observer 1 assessed the consistency of the faecal samples during collection (pig-side).

The faecal samples were placed in transparent plastic containers (height 10 cm, diameter 2 cm) labelled with the pigs' individual ear tag number.

At the end of each visit both Observer 1 and Observer 2 (herd 1+2) or Observer 3 (herd 3+4) assessed the consistency of each faecal sample in the containers (post-collection). They were allowed to manipulate the faecal containers and touch the faeces with a spoon.

A consistency score system with 3 categories (normal, loose and fluid) was used. No further definitions of these categories were given to the observers.

Overall agreement and Cohen's kappa value were calculated for intra-observer agreement (pig-side versus post-collection) for Observer 1. Inter-observer agreement was evaluated in terms of overall agreement, Cohen's kappa value and agreement for each consistency score. The calculations were performed as pair wise comparisons for Observer 1 versus Observer 2 and 3.

Table 1. Inter-observer agreement for assessment of faecal consistency (assessed as normal, loose or fluid).

	Observer 1 versus 2 (n = 148 samples)			Observer 1 versus 3 (n = 116 samples)		
	95% confidence interval			95% confidence interval		
Overall agreement	60.8	52.8	68.3	81.9	73.9	87.8
Normal ¹	85.0	79.4	89.3	94.8	90.1	97.3
Loose ¹	3.8	1.1	13.0	55.8	41.1	69.6
Fluid ¹	18.2	9.5	32.0	68.6	52.0	81.4
Cohen's kappa	0.24	0.17	0.30	0.64	0.55	0.73

¹ Inter-observer agreement for each consistency score

Results

Fifty six faecal samples had to be excluded from the analysis because of missing values or misclassifications.

Overall intra-observer agreement for Observer 1 was 84.4% (95% confidence interval: 80.0 – 88.7) with Cohen's kappa value of 0.72 (95% confidence interval: 0.66 – 0.78). Estimates of inter-observer agreement are displayed in Table 1.

Discussion

We observed a high level of intra-observer agreement between assessments of faecal consistency pig-side versus post-collection in a container. This has implications for the current study because the inter-observer agreement was evaluated post-collection.

The results of this pilot study suggest that assessment of faecal consistency may be subject to variable inter-observer agreement. This implies that observers can have a substantial effect on results of studies where faecal consistency is a key parameter. The results suggest a need for the development of a standardized system for assessment of the consistency of pig faeces.

Only three observers were involved in the current study. This is a small sample of veterinarians and the obtained estimates of inter-observer agreement should be evaluated with caution.

We did not attempt to calibrate the scoring of individual observers prior to the study. It is likely that inter-observer calibration would have increased the level of agreement.

Conclusions Inter-observer agreement may be variable for the assessment of faecal consistency using a simple consistency score.

OBJECTIVE ASSESSMENT OF FAECAL CONSISTENCY IN PIGS PART 2: EVALUATION OF 4 DESCRIPTIVE CATEGORIES WITH TEXT AND PICTURES FOR ASSESSMENT OF FAECAL CONSISTENCY

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Objective

Evaluate intra- and inter-observer agreement for assessment of faecal consistency using a score system with 4 descriptive categories, text and pictures.

Materials and methods

Five observers with at least 5 years of practical veterinary experience were selected at convenience from the same veterinary practice. A diagram with explanations in text and pictures of faeces representing each of the 4 consistency scores was made (table 1).

The diagram was sent by e-mail to the observers 4 days prior to examination. The observers were told to familiarize themselves with the diagram.

At day 1, observer 1 collected 25 faecal samples from each of the 4 consistency categories (n = 100) in a Danish pig herd. The samples were collected in plastic containers (height 7cm, diameter 4.5cm) from pens containing pigs between 2 and 10 weeks post weaning.

At day 2, the 5 observers examined the faecal samples (in the plastic containers) two times in order to assess both intra- and inter-observer agreement. The observers were informed (by observer 1) about the consistency score system immediately before the start of the examination. A large diagram (1 x 0.75m) displaying table 1 was placed in front of the observers during the examinations. The individual observers examined the samples and assessed the consistency scores. They were allowed to manipulate the faecal containers and touch the faeces with a spoon.

Each observer examined the samples in random order. The identification number of the samples was not blinded to the observers.

For each observer the two examinations were performed with an interval of 3.5 to 10 hours.

No inter-observer calibration was allowed prior to the study. During the study the observers were physically separated to avoid calibration.

Results

Intra-observer agreement was only evaluated for the two examinations by observer 1-4. Intra-observer agreement ranged from 0.72 to 0.91 (mean = 0.82) with Cohen's kappa values ranging from 0.61 to 0.88 (mean = 0.76).

Inter-observer agreement was evaluated pair wise for the 5 observers using the first examination of the faecal samples. The inter-observer agreement ranged from 0.61 to 0.90 (mean = 0.73) with Cohen's kappa values ranging from 0.48 to 0.87 (mean = 0.64).

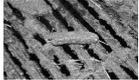
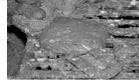
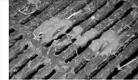
Score	1 Firm and shaped	2 Soft and shaped	3 Loose	4 Watery
Picture				
Texture	Firm. Vary in hardness.	Vary in softness. Like peanut butter	Mush. Often shining surface	Vary form gruel to water.
Shape	Sausage	Vary form sausage shape to small piles	Tends to level with surface. Does not flow through or flows slowly through slatted floors.	Levels with surface. Flows through slatted floors.
In container	Preserves original shape.	Does not flow when container is rotated. Preserves original shape.	Inert when container is rotated. Merges and cover up button of container in most cases.	Flows easy when container is rotated. Merges and cover up button of container.

Table 1. Consistency score with 4 categories, text and pictures

Discussion

This study probably represents a best case scenario without inter-observer calibration when it comes to intra- and inter-observer agreement. All observers were experienced swine veterinarians and were used to examine faecal consistency as part of their job. They were working in the same veterinary practice so any geographic differences in opinion of faecal consistency were eliminated. Further, one would expect that descriptive text and pictures would increase both intra- and inter-observer agreement, because of the possibility to compare the faeces samples with the diagram during the examination.

Conclusion

Four descriptive categories with text and pictures did not eliminate problems of variable intra- and inter-observer agreement in assessment of faecal consistency.

More objective measures of faecal consistency may be more appropriate in research studies where classification of individual samples is important.

**OBJECTIVE ASSESSMENT OF FAECAL CONSISTENCY IN PIGS PART 3:
APPLICATION OF MICROWAVES FOR DETERMINATION OF FAECAL DRY MATTER
CONTENT**

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Objective

The objective was to evaluate repeatability for application of microwaves for determination of faecal dry matter content. Repeatability was evaluated in terms of intra- and inter-serial variation for the microwave procedure.

Materials and methods

Approximately 1 kg of normal faeces was collected from pens containing pigs between 2 to 10 weeks post weaning in a Danish pig herd.

After collection the faeces was divided into 4 samples and homogenised with a spoon. Different amounts of water were added to 3 of the samples to mimic 3 different consistency scores. In this way 4 samples containing a minimum of 250g faeces each were prepared. The 4 samples represented faeces with consistency scores: 1 = firm and shaped, 2 = soft and shaped, 3 = loose and 4 = watery.

From each of the 4 samples 24 subsamples were prepared as follows: The weight of a plastic container (height 7cm, diameter 4.5cm) with an identification number was recorded using a digital weight (precision 0.01g), approximately 4g of faeces was weighed off in the plastic containers and the weight recorded.

A normal household microwave oven with 5 different heating settings and a maximum effect of 700 Watt was used in the study. Paper towels were placed in the bottom of the oven to prevent the bottom of the plastic containers from melting during heating.

The faecal samples were initially heated for 30 minutes at the lowest effect followed by 10 minutes at medium effect. At this point 3 samples were weighed and the weight recorded. All samples were reheated for another 5 minutes at medium effect. The same 3 samples were weighed again. This procedure continued until each of the 3 samples had the same weight 2 times in a row (drying to constant weight). The final weight for each faecal sample was recorded.

The microwave oven was regularly inspected during heating to prevent the faecal samples from burning or boiling.

All procedures were performed by the same person.

Faecal dry matter content was calculated as:

Dry matter = (weight of container and dried faeces – weight of container) / weight of wet faeces.

For each of the 4 consistency scores 8 subsamples were heated together (intra-serial variation). This was performed in 3 replicas (inter-serial variation). Mean, standard deviation (SD) and coefficient of variation (CV) for faecal dry matter content were calculated.

Results

Results are displayed in Table 1.

Discussion

The observed repeatability for the microwave procedure in terms of SD and CV for intra- and inter-serial variation was considered acceptable for determination of faecal dry matter.

The result of the study may only be valid for faeces collected from 6 to 14 weeks old pigs. Faeces from older pigs (i.e. sows) may contain a higher level of faecal dry matter which may influence the intra- and inter-serial variation.

Effect of different operational personnel, weight changes for initial wet faeces, use of different microwave ovens, time between sampling and dry matter determination and effect of overheating may also affect intra- and inter-serial variation. Evaluations of these aspects are in progress along with a study on correlation to freeze-drying.

Conclusion

The microwave procedure represents an easy and fast method for determination of faecal dry matter content. The observed repeatability was considered acceptable.

Table 1.

Dry matter content		Consistency score			
		1	2	3	4
Replica no 1.	Mean	0.251	0.215	0.148	0.075
	SD	0.003	0.007	0.005	0.003
	CV	0.011	0.033	0.036	0.046
Replica no 2.	Mean	0.255	0.213	0.145	0.079
	SD	0.005	0.006	0.002	0.006
	CV	0.019	0.029	0.013	0.072
Replica no 3.	Mean	0.252	0.215	0.148	0.077
	SD	0.003	0.004	0.004	0.005
	CV	0.013	0.020	0.030	0.060
Overall	Mean	0.251	0.215	0.147	0.077
	SD	0.003	0.006	0.004	0.005
	CV	0.012	0.026	0.029	0.061

OBJECTIVE ASSESSMENT OF FAECAL CONSISTENCY IN PIGS PART 4: ASSESSMENT OF FAECAL DRY MATTER CONTENT FOR DIFFERENT FAECAL CONSISTENCY SCORES

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Objective

Assess faecal dry matter content for faeces with different consistency scores. Determine faecal dry matter cut-off values between each consistency score.

Materials and methods

Faecal dry matter content was determined for 100 faecal samples using the microwave procedure described by Pedersen *et al.* (2009b). The faecal samples were collected for a study on evaluation of intra- and inter-observer agreement for 5 observers using a consistency score with 4 categories as described by Pedersen *et al.* (2009a). For each observer the mean faecal dry matter content for each consistency score was determined using analysis of variance. The mixed procedure in SAS version 9.1 was used.

The statistical analysis was performed using the results of the first examination from the 5 observers.

The results of the analysis of variance were used to calculate an overall faecal dry matter mean for each consistency score by taking the average of all observers. The midpoint between the overall mean faecal dry matter content of two consistency scores was used to define cut-off values between the 4 consistency scores.

Results

The mean faecal dry matter content for all faecal samples was 18.0% (range: 6.2% - 28%). The relations between faecal consistency scores and dry matter content are displayed in figure 1 for each of the 5 observers. The analysis of variance showed that for each observer there was a significant difference (p -value < 0.05) in the mean faecal dry matter content for each consistency score. Overall mean faecal dry matter content for each consistency score (assessed by the 5 observers) are displayed in Table 1. Faecal dry matter cut-off values for each consistency score were; score 1: dry matter content > 22.6%, score 2: dry matter content > 18.8%, score 3: dry matter content > 13.1%, score 4: dry matter content <= 13.1%

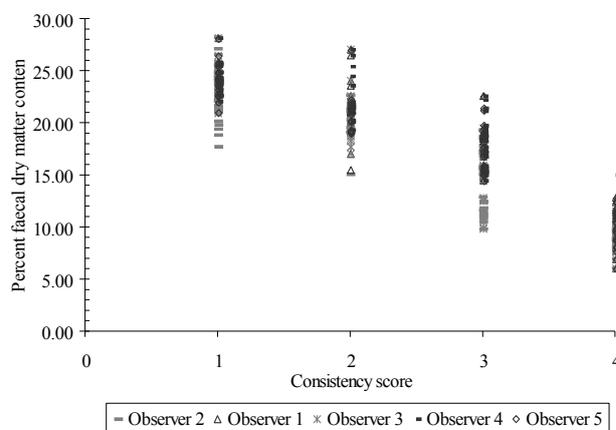
Table 1
Percent faecal dry matter content for 100 faecal samples

Consistency score ¹	Overall mean ²
Score 1 (Firmed and shaped)	24.00
Score 2 (Soft and shaped)	21.20
Score 3 (Loose)	16.40
Score 4 (Watery)	9.70

¹Faecal consistency was assessed by 5 observers.

²Overall mean is average of means for the 5 observers.

Figure 1. Consistency score and faecal dry matter content



Discussion

In this study we used faecal dry matter as an objective measure of the true state of the faecal consistency. Faecal dry matter content may not be the only determinant of faecal consistency. This aspect should be taken into consideration in interpretation of the cut-off values determined in the current study.

The mean faecal dry matter content was significant different between the individual consistency scores for all observers. The small difference between faecal dry matter content for samples scored as 1 and 2 indicate that these two categories may be merged without loss of information in designing consistency categories.

Conclusion

The faecal dry matter content was significant different between different consistency scores. Cut-off values between each consistency score were determined.

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Pedersen, K.S, Stege, H., Nielsen, J.P. 2009b.

CSFV DIVA DIAGNOSTIC USING *PICHIA PASTORIS* AS EXPRESSION SYSTEM

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Introduction & Object

Classical swine fever (CSF) is a highly contagious and often lethal viral disease affecting domestic pigs and wild boars worldwide, including some countries in the European Union (EU).

The causing agent to the disease, CSF virus (CSFV), belongs to the genus *Pestivirus* within the virus family *Flaviviridae*. An outbreak of CSF is economically fatal for the affected country, especially countries with an industrialised pig production. Although outbreaks of CSFV still occur, the disease has been successfully eradicated in domestic pigs in most of the EU member countries due to a strict eradication programme. However, the disease still persists in several wild boar populations around Europe, which acts as a reservoir for CSF.

Efficient live vaccines against CSFV are available for emergency vaccination or prophylactic use, but they elicit the same antibody pattern as those observed in naturally infected animals. This means that infected animals cannot be distinguished serologically from vaccinated animals.

In combination with suitable diagnostic tests, DIVA (Differentiating Infected from Vaccinated Animals) vaccines could be used to monitor and control infections⁽¹⁾.

In theory the DIVA strategy makes it possible to mass vaccinate a susceptible pig population in face of an outbreak to support the control of CSFV or for eradication of the disease in the wild boar population without compromising the serological identification of infected individuals. Therefore large efforts are put into developing new and safe DIVA vaccines along with improved diagnostic tools to convoy the vaccines.

The purpose of this study was to investigate whether it is possible to produce active CSFV structural proteins for further application in DIVA diagnostic assays using the heterologous expression system *Pichia pastoris*. One of the advantages using the yeast *Pichia pastoris* is that it secretes the expressed protein into the growth media for an easy purification.

The CSFV glycoprotein E^{ms} was chosen for the preliminary experiments.

Material & Methods

CSFV cDNA encoding the E^{ms} gene was amplified by PCR, TOPO cloned and after sequencing inserted into the expression vector pGAPZαC before cloning into *Pichia pastoris* strain X-33 using the EasySelect *Pichia pastoris* kit⁽²⁾.

Results

The CSFV E^{ms} gene was successfully amplified by PCR and cloned into the expression vector pGAPZαC. Cloning into *Pichia pastoris* strain X-33 and secreted expression is in progress.

Discussion & Conclusion

The *Pichia pastoris* expression system may be a very useful tool for production of CSFV proteins for diagnostic purposes. It has many of the advantages as higher eukaryotic expressions systems while being as easy to manipulate as *E. coli*. In addition, it is fast, easy and less expensive to use compared to other eukaryotic expression systems and gives a higher yield.

Acknowledgement

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COMPARISONS OF BIRTH WEIGHT, BLOOD LACTATE, BLOOD GLUCOSE IMMUNOGLOBULINS AND SURVIVAL IN NEWBORN PIGLETS OF TWO DIFFERENT DUROC-CROSSBREEDS IN NORWAY

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Introduction

Birthweight and placental function are positively correlated to perinatal survival in the sow (1). Placental properties are genetically equally determined by sow and boar. Colostrum the first day of life is of significant importance for energy and transfer of passive immunity. Colostrum uptake can be indirectly measured on weight gain the first day of life and on the amount of immunoglobulins in plasma, mostly found in the γ globulin fraction and to some extent in the β_2 region (2). The aim of this study was to compare weight, blood parameters and survival in newborn piglets from pure Duroc boars with Landrace-Duroc hybrid boars.

Materials and methods

Farrowing and litters of 18 Landrace-Yorkshire sows in one farm were observed over three weeks. Nine of the sows had been inseminated with Landrace-Duroc (LD) semen and nine with pure Duroc (DD) semen. The sows were individually loose-housed in similar sized pens from three weeks before farrowing. Gestation number varied from one to eight. All sows were fed traditional lactation-feed and small amounts of hay. Only piglets that were observed at birth were included in the study and immediately bloodpunctured in the jugular vein for measurement of blood glucose and -lactate, using fullblood on a handheld glucometer (Bayer Breeze 2) and i-STAT 200 portable clinical analyser (Abbott Laboratories, Illinois, USA). Birth weight, stillbirth and litter size were recorded. The same piglets were again weighed 24 hrs +/-10 later and bloodpunctured. EDTA blood was kept at room temperature until arrival at Central Laboratory, Norwegian School of Veterinary Science, Oslo, Norway, the next morning for measurement of plasma proteins (Sebia, Capillarys 2). Cross fostering within the same group were, if necessary, performed after 24 hours. Statistical analyses were made by JMP 8, using simple Chi-square test and variance analyses.

Results

Of total 261 observed newborn piglets, 8.8 % were stillborn. There was no significant difference in stillbirth or litter size ($15.5 \pm SE 0.21$) between Group LD and DD. There was a highly significant difference ($P < 0.01$) between the two groups in birth weight, blood lactate, weight gain at day 1 and plasma proteins. It was no significant difference in blood glucose between the two groups.

Group		Birth weight kg	Blood lactate mmol/L	Blood glucose mmol/L	Weight gain, day 1 kg	TP day 1 g/L	β_2 -glob day 1 g/L	γ -glob day 1 g/L
LD	mean	1.32	6.91	2.90	0.075	52.0	8.0	20.4
	S.E.	0.03	0.43	0.16	0.01	0.94	0.23	0.71
	n	115	66	107	104	102	102	102
DD	mean	1.49	5.43	2.57	0.124	59.8	11.3	24.6
	S.E.	0.03	0.38	0.17	0.01	0.96	0.24	0.72
	n	116	82	100	99	98	98	98

Table 1: Means of birth weight, blood lactate, blood glucose, weight gain day 1, total plasma proteins (TP), β_2 - and γ -globulins in Group LD and DD.

Discussion and Conclusion

The observed differences in this study are likely to be caused by the sire. Results indicate that the increased blood lactate in Group LD is not elevated due to increased blood glucose or birth weight, but may be caused by stress due to prepartum oxygen-deficit. Increased immunoglobulin levels after one day of life indicate higher intake of colostrum or better immune status in the sow. Increased weight gain indicate the former.

Preliminary analyses from this study show that piglets with a Landrace-Yorkshire hybrid mother have higher weights and lower blood lactate levels at birth when the boar is pure Duroc, compared to a Landrace-Duroc hybrid boar. Also, weight gain and uptake of immunoglobulins is higher after one day in the pure Duroc group.

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POSSIBLE PATHOGENIC INTERPLAY BETWEEN CHLAMYDIA SUIS, CHLAMYDOPHILA ABORTUS AND PORCINE CIRCOVIRUS TYPE 2 ON AN ESTONIAN PIG PRODUCTION PLANT

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Objectives.

In 1996, a new infectious disease in specific-pathogen-free (SPF) swine herds in Western Canada was identified and reported (Clark, 1997; Harding & Clark, 1997). The disease was caused by a DNA virus referred to as porcine circovirus 2 (PCV2). Chlamydial infections in pigs have been known to occur in Europe since 1955 (Willingan & Beamer, 1955). The paper deals with the occurrence of chlamydial disease in boars, sows and gilts and concurrent PMWS in weaned piglets on a large Estonian pig production plant.

Materials and methods.

Conjunctival, vaginal and faecal swabs of boars, sows and gilts were examined by culture, a direct immunofluorescence test on conjunctival smears and a Chlamydiaceae-specific micro array. Boar sera were also examined by use of a Chlamydiaceae major outer membrane protein (MOMP)-based recombinant antibody ELISA and a complement fixation test. Lymph nodes of piglets showing PMWS were examined for the presence of PCV2 by use of immunohistochemistry and sow sera were examined by a PCV2 immunoperoxidase monolayer assay.

Results and discussion.

Chlamydia suis DNA was detected in conjunctival swabs of boars, sows and gilts, but also in faeces of boars and sows. *Chlamydomphila abortus* DNA was found in semen and in sow eyes. DNA was demonstrated by micro arrays. Boar sera were negative by CFT, but all examined sera were positive (1/960 to 1/3840) by the recombinant MOMP-ELISA. Chlamydiosis was characterized by reproductive failure and conjunctivitis. Piglets were not examined for Chlamydiaceae as eye problems were not observed. However, as *Chlamydia suis* was present in faeces of sows, piglets most probably became infected which could have led to the PMWS outbreak in weaned piglets. Piglets showed wasting, respiratory signs, diarrhoea, enlargement of lymph nodes and increased mortality (10%). PCV2 was detected in the lymph nodes of piglets by immunohistochemistry and PCV2 antibodies were demonstrated in all 10 examined sow sera using an immunoperoxidase monolayer assay (103 to 105).

Boars were treated with tetracyclines and vitamin supplements and the situation improved but the 'problem' was not completely solved as some boars still get the sickness, but not as severe, and not in so many boars, as before. At present, new boars stay in quarantine for 6 weeks instead of 4. Sows and gilts no longer show clinical evidence of chlamydial infections. The plant started to vaccinate all weaned piglets with a PCV2 vaccine (Suvaxyn PCV2, Fort Dodge) and all new boars with the Porcilis Glässer (*Haemophilus parasuis*) vaccine (Intervet). Additionally, all older boars are twice a year injected with the Glässer disease vaccine. The PMWS problem in weaned piglets is solved.

Conclusion.

The study demonstrates the impact of a co-occurrence of *Chlamydiaceae* and PCV2. Both pathogens can be present in healthy pig herds, but might trigger each other's pathology.

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SEROCONVERSION AND PCV2 VIREMIA FOLLOWING VACCINATION WITH CIRCOVAC (MERIAL) AND CIRCOFLEX (BOEHRINGER INGELHEIM) VACCINES

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Objective:

Vaccines against PCV2 provide good tools against PCVD. Vaccination helps to control PCV2 viremia and virus shedding following the natural infection. Also, the vaccines can be used in replacement gilts of seronegative or unknown status regarding PCV2 to protect against reproductive failure following natural infection during gestation. The objective of this study was to compare viremia and seroconversion in pigs from a herd subclinically infected with PCV2, vaccinated with two different vaccines against PCV2.

Materials and methods:

The pig herd U is a farrow to finish farm with 700 sows. In 2006 acute outbreak of PCVD in fattening pigs occurred. Since December 2007 vaccination of pigs at 55 days of age with ½ dose of Circovac have been applied and PCVD was successfully controlled. For this study 60 piglets were divided into groups of 20 and vaccinated at 73 days of age with 1 dose of Circoflex (Boehringer Ingelheim) (group D) or ¼ dose of Circovac (group F). Group C served as control. Pigs from the experimental and control groups were comingled. Serum samples collected on 4 to 22 weeks of age were tested for the presence of PCV2 antibodies with Ingezim Circovirus ELISA (Ingenasa) and for the presence of PCV2 DNA by real time Q-PCR.

Results:

It was found that at 4 weeks of age only 11.7% of piglets from all groups were seronegative for PCV2. In the following weeks the proportion of seronegative pigs gradually increased and at the time of vaccination 96.6% of pigs had no detectable antibodies for PCV2. Interestingly, one pig seropositive at the time of vaccination (15C) was viremic from 28 days of age and seroconverted at 56 days of age. This pig died before 140 days of age. There was substantial difference in the seroconversion profiles following vaccination with the two vaccines. At 11 days post vaccination all pigs immunized with Circovac were seronegative while two pigs vaccinated with Circoflex were seropositive. However, one of those (11D) was found to be infected yet before vaccination and the second (17D) became viremic at 11 days post vaccination. So, none of these two seroconversion events could be attributed to the vaccination but rather to natural infection. At 25 days post vaccination 38.9% of Circovac vaccinated pigs seroconverted with high levels of IgM antibodies while none of Circoflex vaccinated pigs did, except for those that were earlier infected. At age of 112 days, so 39 days post vaccination, about half of pigs from vaccinated and control groups became viremic. In the following weeks the proportion of viremic pigs fluctuated but always the highest value was recorded in the control group. Also the mean Ct values for the pigs from the control group was much lower than in the vaccinated groups.

Discussion:

In the farm U most of the pigs are protected against PCV2 infection until about 112 days of age. However, very small proportion of individual pigs can be infected earlier. Of 7 pigs seronegative at 28 days of age, 3 became infected before transferring to the fattening units. One of them, apparently infected before weaning, remained viremic for the rest of its life. This pig died before 140 days of age but the cause of death was not related to PCV2 or other infection. Previous studies proved high efficacy of vaccination against PCVD. This experiment indicated that the vaccines against PCV2 can also be effective in decreasing viremia in pigs from subclinically infected herds. Preliminary results indicated that also virus shedding with feces was limited by the vaccination. At 154 days of age around 77% of control pigs shed PCV2 with feces while only 59% of Circovac vaccinated and 53% of Circoflex vaccinated pigs did. Interestingly, serological response due to vaccination was detected only in Circovac vaccinated pigs. Circoflex vaccinated pigs seroconverted following natural infection but unlike in pigs from the control and Circovac vaccinated, where majority of pigs produced high levels of IgM for extended time, seroconversion was restricted to IgG. Only in 3 samples from 3 pigs vaccinated with Circoflex IgM antibodies were detected.

Conclusions:

Vaccination against PCV2 of 73 days old seronegative pigs does not delay natural infection but it limits viremia and virus shedding in feces.

Circovac and Circoflex vaccines differ in seroconversion profiles post vaccination and following infection of the vaccinates which could be attributed to their different antigen and adjuvant composition.

IDENTIFICATION OF PORCINE CIRCOVIRUS TYPE 2, *LAWSONIA INTRACELLULARIS* AND *BRACHYSPIRA HYODYSENTERIAE* IN PIGS WITH DIARRHOEA

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Introduction

The aim of the study was to analyze the presence of porcine circovirus type 2 (PCV2) in cases of antibiotic non-responsive diarrhoea and to evaluate the possible role of the virus in development of enteritis in pigs. For differential diagnosis identification of *Lawsonia intracellularis* (*L. intracellularis*) and *Brachyspira hyodysenteriae* (*B. hyodysenteriae*) was performed.

Material and methods

Internal organs and feces were collected from 76 pigs, 5 – 19 weeks old, from 50 farrow-to-finish farms, PMWS-positive or PMWS-suspected. Sections of lymph nodes and intestines (ileum, caecum and colon) were analyzed for presence of PCV2 DNA by in situ hybridization test (ISH) (2). They were also hematoxylin-eosin (HE) stained for standard histopathological examination. Additionally, fecal samples were tested for presence of *B. hyodysenteriae* and *L. intracellularis* by PCR (3).

Results

In samples from 37 pigs, 10 – 17 weeks old, large amounts of PCV2 DNA, typical for PMWS, were detected in lymph nodes by ISH. In this group, in samples from 19 pigs, PCV2 was also found in abundant amount in samples of ileum. The remaining 18 pigs, PCV2-positive in lymph nodes, were negative in ileum. In samples from only 1 animal lymph nodes were negative for PCV2 in ISH, but virus was detected in considerable amount in ileum. In HE stained sections of lymph nodes histopathological lesions characteristic for PMWS were identified. Similar lesions were observed in PCV2-positive samples of ileum. Seventy samples of feces were negative in PCR for *B. hyodysenteriae* and *L. intracellularis*. DNA of *L. intracellularis* was found in feces from 3 pigs and mixed infection caused by both bacteria was detected in 3 animals.

Discussion

According to the obtained results in PMWS-affected pigs similar lesions could be observed both in lymph nodes and in ileum and they correlate with clinical outcome of disease. Also, it was found that presence of PCV2 in ileum could be correlated with diarrhoea in PMWS-free animal, which confirms that the emergence of this virus as an intestinal pathogen may represent a new phenomenon (1). In the animals negative for *B. hyodysenteriae* and *L. intracellularis* and PCV2 other causative agents of diarrhoea should be considered. Further investigation on larger number of samples needs to be performed to confirm the role of PCV2 as an etiological agent of diarrhoea in pigs.

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BENEFITS OF PCV2 VACCINATION IN A CASE OF ACUTE PCVD COMPLICATED BY APP CO-INFECTION

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Objective

The negative impact of Porcine Circovirus Diseases (PCVD) in pig production has been confirmed in different countries. In PCVD affected farms the percentages of mortality and runts are increased together with a reduction of growth rate whatever form of the disease is observed (1). In 2008 Ingelvac CircoFLEX[®] was the first PCV2 vaccine for piglets that became available in the EU. The objective of this study was to evaluate the benefits of the vaccination with Ingelvac CircoFLEX[®] in a multisite farm, suffering from acute PCVD and severe outbreaks of APP. The farm was producing heavy pigs, with a minimum slaughter age of 9 months.

Materials and methods

The study was carried out in a 3-site 400-sow herd. Piglets were moved to site 2 at about 4 weeks of age and to site 3 at 12 weeks. The animals suffered from the acute form of PCVD, during the nursery production phase (site 2), and after the transfer to the site-3 severe outbreaks of pleuropneumonia due to *Actinobacillus pleuropneumoniae* (APP) constantly recurred. Pigs were vaccinated at weaning with a single dose (1 ml) of Ingelvac CircoFLEX[®] (n=381) or left unvaccinated as controls (n=397). Vaccinated and control pigs were allocated in the same barns and kept in separate pens. In each group 10 individually identified randomly selected pigs were bled at weaning (T0), 4 weeks later (T1), at the peak of the clinical disease (T2) and before slaughter (TS) (approximately 9 month after vaccination). Sera were tested by qPCR to determine the course of PCV2 infection. Mortality and runts data were recorded per group in site 2 and 3. Individual carcass weight was recorded in all slaughtered pigs. Mortality and runts were compared using Chi-square test and slaughter weight differences were analysed with a two-sample t-test, (SAS System, SAS Inst., Cary, North Carolina, v 8.2).

Results

Both vaccinated and control pigs were negative at qPCR at weaning (T0). Four weeks later (T1) control pigs were PCV2-positive even if not showing any clinical signs, while vaccinated were negative. At 10 weeks of age (T2 -the peak of the acute PCVD) all controls were massively positive (qPCR titres: min 3.5 – max 7.8 log₁₀ DNA genomic copies/ml of serum) while only few vaccinated pigs (30%) showed low titres (min 3.1 – max 4.5 log₁₀ DNA genomic copies/ml). No pigs were viremic at TS (before slaughter). After the transfer to site 3 both groups suffered from an acute APP outbreak, causing a different mortality figure- in vaccinated vs controls. Wean-to-finish mortality and runts were significantly reduced in the vaccinated group compared to the controls (Tab. 1).

Tab. 1. Mortality and runts.

Period	Parameter	Circo-FLEX	Control	p-value
Site 2	Mortality, %	2.1	8.8	< 0.0001
	Runts, %	0.5	0.8	Ns
Site 3	Mortality, %	7.4	28.4	< 0.0001.
	Runts, %	4.9	9.8	< 0.02
Total	Mortality, %	9.2	34.3	< 0.0001.
	Runts, %	5.2	9.6	< 0.03

P-values ≤ 0.05 indicate a statistical significant difference, p-value > 0.05 not significant (n.s.).

Carcass weight of vaccinated pigs was 1 kg heavier compared to controls (130.3 vs 129.3 kg). The average slaughter age differed by 15 days between the two groups (CircoFLEX: 285 days, control: 300 days, p<0.001).

Discussion and conclusion

The present field study demonstrates that Ingelvac CircoFLEX[®] vaccination can provide significant benefits in a multisite production system, suffering from a severe co-infection, where pigs are slaughtered with a minimum age of 270 days.

The impact of the APP infection was lower in PCV2 vaccinated animals as demonstrated by a significantly reduced mortality and number of runts in site 3.

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LONG DURATION OF PROTECTION OF INGELVAC CIRCOFLEX IN PIGS REARED UP TO 9 MONTHS OF AGE

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Objective

Porcine Circovirus Diseases (PCVD) are responsible for significant economical losses to the pig industry. In 2008 Ingelvac CircoFLEX[®] was the first PCV2 piglet vaccine that became available in the EU. In registration trials it had been shown that the vaccine provides protection for at least 17 weeks after vaccination (1). The objective of this study was to evaluate if vaccination with Ingelvac CircoFLEX[®] can provide protection through to slaughter in pigs that are produced under the specific requirements for Parma ham production, with a minimum slaughter age of 9 month.

Materials and methods

The study was carried out in a single-site 700 sow farm. Piglets were weaned and transferred to the nursery at about 4 weeks of age. At 12 weeks of age pigs entered the growing unit and were transferred to the finishing unit at 18 to 20 weeks of age. Pigs were vaccinated at weaning with a single dose (1 ml) of Ingelvac CircoFLEX[®] (n=261) or left unvaccinated as controls (n=261). Vaccinated and control pigs were kept in separate pens but in the same room. In each group 10 randomly selected pigs were bled at weaning, 4 weeks later and before slaughter (~ 9 month after vaccination). Sera were tested by qPCR to determine the course of PCV2 infection.

Mortality and runts were recorded per group in nursery, growing and finishing periods. Runts were defined as those pigs sold pre-slaughter as underweighted pigs or culled because of severe disease. Individual carcass weights were collected from all slaughter pigs.

Mortality and runts were compared using Chi-square test and slaughter weight differences were analysed with a two-sample t-test, (SAS System, SAS Inst., Cary, North Carolina, v 8.2).

Results

Vaccinated and control pigs were negative in qPCR at weaning. Control pigs were PCV2-positive 4 weeks after weaning and before slaughter. This was in line with the clinical observation with a peak of clinical signs being observed at 55 days of age and in the last weeks before slaughter. In contrast, all samples from vaccinated animals were negative in qPCR throughout the trial. Wean-to-finish mortality and runts were significantly reduced in the vaccinated group compared to the controls (Table 1).

Period	Parameter	Circo-FLEX	Control	p-value
Nursery	Mortality, %	0.8	3.8	< 0.02
	Runts, %	1.9	9.6	< 0.001
Growing	Mortality, %	2.4	2.7	n.s.
	Runts, %	2.4	2.2	n.s.
Finishing	Mortality, %	1.2	7.9	< 0.001
	Runts, %	3.3	2.8	n.s.
Total	Mortality, %	4.2	12.6	< 0.001
	Runts, %	7.3	13.8	< 0.02

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

The average slaughter age was different by about 1 day between the two groups (Ingelvac CircoFLEX[®]: 303 days, Control: 304 days). Vaccinated pigs had a 5.1 kg heavier carcass weight on average than control animals (140.6 vs 135.5 kg, $p < 0.01$).

Discussion and conclusion

Clinical efficacy of Ingelvac CircoFLEX[®] was confirmed by a significant reduction in mortality and number of runts in vaccinated animals compared to controls and a significant increase in carcass weight.

In the field condition of the trial PCV2 natural exposure was present until the end of the finishing production phase, as shown by positive qPCR results in the control animals. The present field study demonstrates that Ingelvac CircoFLEX[®] can provide protective immunity against PCV2 infection for up to 9 months after vaccination.

References

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THE EFFECT OF FEEDING STRATEGY AND PARITY NUMBER ON FEEDING CAPACITY, WEIGHT LOSS AND PRODUCTION IN NORWEGIAN LOOSE HOUSED HYBRID (LY) SOWS.

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Objective

The main objective of these trials was to investigate the effect of two different feeding strategies and Parity number on feeding capacity, weight loss and production in Norwegian LY-sows.

Materials and methods

Data were obtained from 98 loose housed Norwegian LY-sows from two different commercial swine herds. Sows were fed using a pelleted lactation feed containing 8.48 MJ NE/kg feed and 8.26g Lysin/kg feed. Feed was increased with 330grams per feeding every other day over a period of two weeks. Sows were fed twice a day in week one, then three times a day. During transition from two to three feedings, the feeding level was adjusted to avoid an increase of 50% over night. From week three sows were fed either ad libitum or continued on controlled feeding. Within 12-36 hours after parturition (day 1) number of live born and still born were recorded along with litter weight and weight of the sow. Sow and litter were weighed again the day before weaning and day of weaning, respectively. Average length of lactation was 33.6 days. Daily feed consumption was recorded (sows), and piglets were given creep feed from week two. The data was analysed using Proc GLM in SAS 9.1 2001-2003 edition. Feed consumption, weight loss and litter weight gain were adjusted for length of lactation. Fixed effects included feeding strategy, herd and parity number.

Results

Table 1: Overview of the raw average per group according to parity number

Parity	n	Group average				ADLG ²⁾ ± SD (Kg)
		Weight day 1 ± SD (Kg)	ADFC ¹⁾ ± SD (Kg)	Weight loss ± SD (Kg)	Litter size at weaning ± SD	
1	22	221.5 ± 14.3	7.3 ± 1.1	39.5 ± 13.9	11.7 ± 1.1	2.9 ± 0.5
2	10	253.3 ± 28.3	8.2 ± 0.7	40.2 ± 16.4	12.2 ± 0.9	3.4 ± 0.5
3-7	66	292.0 ± 28.5	8.8 ± 1.1	24.6 ± 19.6	11.6 ± 1.5	3.2 ± 0.7

Table 2: LS means for the effect of feeding strategy on ADFC, Weight loss and ADLG

Feeding strategy	n	LS means ³⁾		
		ADFC ¹⁾ ± SE	Weight loss ± SE	ADLG ²⁾ ± SE
Ad libitum	47	8.5 ^a ± 0.12	1.1 ^a ± 0.07	3.08 ^a ± 0.06
Controlled	51	8.2 ^b ± 0.11	0.9 ^b ± 0.06	3.24 ^b ± 0.06

Table 3: LS means for the effect of parity number on ADFC, Weight loss and ADLG

Parity	n	LS means ³⁾		
		ADFC ¹⁾ ± SE	Weight loss ± SE	ADLG ²⁾ ± SE
1	22	7.87 ^a ± 0.16	1.06 ^a ± 0.11	3.09 ^a ± 0.08
2	10	8.40 ^b ± 0.22	1.10 ^a ± 0.11	3.23 ^a ± 0.11
3-7	66	8.68 ^b ± 0.09	0.82 ^b ± 0.05	3.16 ^a ± 0.04

¹⁾ Average daily feed consumption of the sow, ²⁾ Average daily litter gain, ³⁾ LS means with different lettering differ significantly from each other

Discussion and conclusion

Sows fed ad libitum have a higher feed consumption ($p < 0.04$), but loose more weight ($p < 0.02$) and wean smaller piglets ($p < 0.02$) than the controlled group. This might be due to larger individual difference in the ad libitum group (more extremes). Loss of appetite was rarely seen amongst the sows that continued on controlled feeding. First parity sows have lower ADFC than elder sows ($p < 0.001$), and first and second parity sows loose numerically more weight than elder sows ($p < 0.08$). The feeding capacities of the sows increase with increasing parity number ($p < 0.001$) but large individual differences are seen. There was also an effect of herd ($p < 0.0001$) on ADFC, weight loss and ADLG, which have been explored in more recent trials where we investigate the effect of water access in the fed trough on the feed utilization of sows.

FOLLOW-UP STUDY OF THE DEVELOPMENT OF ANTIBODIES AGAINST *CAMPYLOBACTER* SSP. IN PIGLETS IN A FARROW-TO-FEEDER HERD.

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Objective

Maternal *Campylobacter* strains are the primary source of infection for piglets within their first days of life [1] and the prevalence may rise to 100 % by the time of weaning [6]. Serological investigations of *Campylobacter* infections in pigs are rare. The purpose of the study was to demonstrate the development of antibodies against *Campylobacter* in piglets over a period of seven weeks by using an in-house ELISA.

Animals, Materials and Methods:

Five sows and two to three piglets of the litters were examined over a period of seven weeks. Blood and faeces were taken within four days after birth (SD 1) from the sows. 14 suckling pigs were tested within four days p.p. by taking blood (SD 1). Afterwards, the signed piglets were examined after seven days (SD 2), one day before weaning (SD 3) and two weeks later (SD 4) in the nursery unit by taking blood samples and faecal swabs. Swabs were stored in Cary-Blair-Medium (BBL Culture Swab, Cary-Blair Agar, Becton Dickinson, Heidelberg, Germany).

Multiplex PCR-method: Template DNA was prepared out of faeces using the PSP[®] Spin Stool DNA Kit (Invitex, Berlin, Germany) and *Campylobacter* was detected by PCR according to Persson and Olsen [4].

Serological investigation: *C. coli* major outer membrane protein (MOMP) [7] was cloned and expressed using the pTrCHis Topo[®] TA Expression Kit (Invitrogen, Carlsbad, CA, U.S.A.). Protein concentration was determined using the Micro BCA[®] Protein Assay (Pierce, Rockford, U.S.A.) and Polysorp[®] microtiter plates (Nunc, Roskilde, Germany) were coated. OD % values were calculated as percent positivity (PP) values using a defined positive serum from a *Campylobacter* infected pig as reference.

Results

C. jejuni was not identified neither from the faeces from the sows nor from the piglets. From the time of weaning, all piglets excreted *Campylobacter* spp., but isolation of *C. coli* succeeded mostly at the time of weaning (SD 3: 85.7 %). Strains, which could not be identified as *C. jejuni* or *C. coli*, predominated at the nursery stage (SD 4: 57.1 %). While the isolation rate of *Campylobacter* from the faeces increased up to the time of weaning, serologically the portion of maternal antibodies decreases steadily between the first three sampling days, but stagnated between SD 3 and SD 4 and the first increasing antibody titers were observed.

Discussion

Neither maternal antibodies nor antibody response at the later stage of life seem to limit colonisation the gut. Bacteraemia in pigs is not documented. Therefore, circulating antibodies may play only a role in limiting invasion as assumed in humans [2]. Regarding the high bacteriological prevalence of *Campylobacter* in pig herds, constant exposure leads to permanent colonisation of the gut [3], but it will also lead to repeated challenge with *Campylobacter*, which in turn induces permanent antibody responses. Nonetheless, bacteriological and serological results did not correlate statistically, this also being determined when using immunoblot method [5]. The low specificity of the test was attributed to the lack of negative samples, serum of gnotobiotic piglets served as negative control, and to the discontinuous excretion of the agent. Sensitivity and specificity of the established ELISA as well as a positive-negative cut-off value have not been determined yet, but will face the same difficulties.

Conclusions

Although bacteriological and serological investigation came to the same result of a high *Campylobacter* prevalence in the farrow-to-feeder herd, serological testing is preferable to bacteriological methods on the basis of practicality, time-saving aspects and costs. After evaluating the ELISA-test it could be possible to categorise pig herds according to their prevalence. Based on this knowledge, epidemiological investigations on risk-factors might be possible.

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HEALTH BONUS AFTER TRANSITION TO 4- OR 5-WEEK BATCH MANAGEMENT SYSTEMS

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Introduction and Objective

Sow batch management systems (BMS) become more popular because of advantages in labour planning, batch sizes of piglets, 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 the 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 BMS.

Materials and Methods

Ten wean to finish pig farms, changing from a conventional 1-week management system to a 4- or 5-week BMS were included. The average farm size was 227 sows (min. 130, max. 500 sows). Samples were collected before, 6 to 8 months and 2 years after transition to the BMS. The type of samples and the different analyses are described in Table 1.

Table 1. Sampling schedule before and after transition to a 4- or 5-week BMS

Sample type	Animal category	Type of analysis**	# of samples
Blood/serum	Gilts	<i>Lawsonia</i> , PRRSv, <i>M. hyo</i>	10
	Sows (> 3 litters)	<i>M. hyo</i>	10
	Piglets (10 wk)	<i>Lawsonia</i> , PRRSv, App	10
	Grower pigs (45 kg)	<i>Lawsonia</i> , PRRSv, App, <i>M. hyo</i>	10
	Finisher pigs (> 80 kg)	<i>Lawsonia</i> , PRRSv, App, <i>M. hyo</i>	10
Nasal swabs	Piglets (6 wk)	PCR DNT*	12
	Piglets (10 wk)	PCR DNT*	12
	Grower pigs (45 kg)	PCR DNT*	12
Mixed faeces (faeces of 5 animals)	Grower pigs (45 kg)	PCR <i>Brachyspira</i> spp.	1
	Finisher pigs (> 80 kg)	PCR <i>Brachyspira</i> spp.	1

* DNT = *Pasteurella multocida* dermonecrotic toxine; ** ELISA on serum samples: *Lawsonia* – Bioscreen Enterisol® Ileitis ELISA, PRRS – Idexx HerdChek PRRSv Ab test, *M. hyopneumoniae* – Idexx HerdChek *M. hyopn.* Ab test and *A. pleuropneumoniae* – Idexx Chekit – App – ApxIV ELISA

Results

All nasal swabs were negative for *Pasteurella multocida* DNT during the entire study period and no clinical signs of atrophic rhinitis were observed. All faecal samples were negative for *Brachyspira hyodysenteriae*. Results of serological analyses are shown below (Table 2).

Table 2. Results of serological analyses, expressed as percentage positive animals before and after transition. (green punt)

Disease	Animal group	Before (1)	6-8 months (2)	After 2 years (3)	% change (1-2)	% change (1-3)
PIA	Gilts	90	96	90	-7%	0%
	Piglets	22	5	5	77%	77%
	Grower pigs	52	21	32	60%	38%
	Finisher pigs	64	72	70	-13%	-9%
PRRSv	Gilts	90	81	83	10%	8%
	Piglets	54	46	61	15%	-13%
	Grower pigs	80	93	72	-16%	10%
	Finisher pigs	80	94	89	-18%	-11%
<i>M. hyo.</i>	Gilts	51	46	49	10%	4%
	Sows	16	18	36	-13%	-125%
	Grower pigs	51	17	32	67%	37%
	Finisher pigs	77	45	46	42%	40%
<i>A. pleuropn.</i>	Piglets	55	45	65	18%	-18%
	Grower pigs	40	22	35	45%	13%
	Finisher pigs	74	59	51	20%	31%

Discussion and Conclusions – The results clearly show some particular effects of a transition to a 4- or 5-week BMS on the general health status of the pig farms. Transition to BMS clearly postpones or slows down the seroconversion for *Lawsonia*, *M. hyopneumoniae* and *A. pleuropneumoniae*. This may allow the farmer to vaccinate the pigs before they come in contact with the pathogen and allows the pigs more time to build up immunity. Weaning age, maternal protection and transmission of pathogens from the sows to the piglets determine the speed and level of horizontal transmission within the batch. The level of isolation of the batches from each other then determines the horizontal transmission between subsequent batches. In conclusion, the results show a clear health bonus with the introduction of a 4- or 5-week BMS.

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RECENT ANTIMICROBIAL SENSITIVITY DATA OF BRACHYSPIRA HYODYSENTERIAE IN BELGIUM

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Introduction and Objectives

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 *Brachyspira hyodysenteriae* AMR in Belgium. Therefore, we studied (project 'Veepeiler-varken' funded by the Sanitary Fund) the susceptibility of recently isolated strains of *Brachyspira hyodysenteriae* to the pleuromutilins tiamulin and valnemulin.

Materials and Methods

Strains of *Brachyspira hyodysenteriae* (n = 45) were isolated from routine diagnostic samples during 2008 and immediately after isolation, antimicrobial susceptibility testing was performed. Besides the field isolates, available reference *Brachyspira* strains were also run into the test (2). Susceptibility testing was performed as previously described (1, 2). The 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

The reference strains of *B. hyodysenteriae* were far more susceptible to valnemulin (MIC ≤ 0.03 and 0.06) than to tiamulin (MIC = 0.06 and 1). Results of the field isolates of the different *Brachyspira hyodysenteriae* strains are given in Table 1 for tiamulin and valnemulin. Minimal inhibitory concentrations for 50 (MIC₅₀) and 90% (MIC₉₀) of the strains tested are also given in Table 1.

Table 1. Minimal inhibitory concentrations and MIC₅₀ and MIC₉₀ for *B. hyodysenteriae* strains isolated in 2008

Antimicrobial agent	Number of strains with MIC (µg/ml) of										MIC ₅ 0	MIC ₉ 0
	≤ 0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8		
Tiamulin	1	3	8	8	4	2	3	4	2	10	4	8
Valnemulin	15	4	7	2	2	1	1	6	1	6	0.12	> 8

Discussion and Conclusions

In comparison with previous studies (1,3), MIC₅₀ and MIC₉₀ were significantly increased for *B. hyodysenteriae*. Considering that isolates with MIC ≥ 1 µg/ml should be regarded as not responding to therapy *in vivo*, 21 out of 45 strains of *B. hyodysenteriae* were resistant to tiamulin (1,3). For valnemulin, the number of resistant strains was lower with 15 *B. hyodysenteriae* strains resistant out of the 45 strains tested. These results reflect the field data on therapeutic first choice use of tiamulin in the treatment of swine dysentery. The fact that an increasing number of strains reaches the highest concentrations tested for both pleuromutilins is a serious concern for the Belgian pig industry. Farms should be aware to further improve their external or internal biosecurity measures in order to omit any entrance of *B. hyodysenteriae* strains on the farm. In conclusion, antimicrobial resistance to pleuromutilins has changed towards more resistance and higher MIC₅₀ and MIC₉₀ values as compared to previous data, especially for tiamulin which is considered first choice antibiotic in the treatment of swine dysentery in Belgium.

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HYGIENOGRAMS FOR THE EVALUATION OF CLEANING AND DISINFECTION PROTOCOLS IN PIG FACILITIES

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Introduction and Objectives

Cleaning and disinfection (C&D) is well established in the daily routine of the Belgian pig herds. Nevertheless, correct application of C&D protocols is not evident. The use of hygienograms to control and evaluate C&D protocols on pig farms – as already widely used in poultry (1) – should be promoted. Therefore, a study was conducted to evaluate a hygienogram scoring system following C&D according to the farmers' routine procedures and a protocol as prescribed by CID.

Materials and Methods

A comparative study between the farmers' routine protocol and an established C&D protocol, according to prescriptions of CID Lines, was performed on six pig farms in Flanders. Two comparable compartments (farrowing house, nursery or fattening stable), emptied at the time of the study, were subjected to one of both protocols. The standard C&D protocol by CID Lines consisted of the steps described in Table 1. The farmers' routine protocol differed from farm to farm.

Table 1. Standard cleaning and disinfection protocol of CID Lines

Application	Product	Description	Dose	Contact time
Dry cleaning	-	-	-	-
Soaking	Water	Low pressure	-	> 6 h
Main washing	Water	100-150 bar; 20-30 L/min	-	-
Foaming	BIO CID S	Alkaline product	2 %	10-20 min
2 nd washing	Water	100-150 bar; 20-30 L/min	-	-
Flushing	Water	Low pressure; High flow	-	-
Disinfection	VIROCID	Concentrated broad spectrum	0.5 %	> 10 min
Closure	-	-	-	min. 24 h
Thermofogging	Kickstart	Concentrated broad spectrum	1L on 5L H ₂ O per 1000 m ³	> 10 min

After C&D, hygienograms were taken from six different places in the compartment. All places were sampled twice per compartment, resulting in 12 hygienograms per compartment. Hygienograms are taken through a 10 s contact with the surface, followed by an incubation at 37°C for 24 h. In addition, a positive and negative control are taken. Colony counts (cfu/plate) are performed after 24 h, followed by scoring, according to the following schedule: score 0 = 0 cfu/plate; score 1 = 1-40 cfu/plate; score 2 = 41-120 cfu/plate; score 3 = 121-400 cfu/plate; score 4 = > 401 cfu/plate and score 5 = uncountable.

Results

The results are presented in Table 2. The global score clearly shows that C&D according to CID lines protocol results in a better score as compared to the usual farmers' procedure. Feeders and floors scored highest, especially in the facilities cleaned and disinfected with the farmers' protocol.

Table 2. Results of comparative evaluation of two cleaning and disinfection protocols

	COMP 1 - CID				COMP 2 – farmer				Average
	cfu	cfu	score	score	cfu	cfu	score	score	
Pen wall	3	0	1	1	3	9	1	1	1
Floor	36	11	1	1	99	105	2	2	1.5
Slat	14	26	1	1	50	39	2	1	1.25
Outer comp. wall	25	45	1	2	23	18	1	1	1.2
Upper wall part	9	29	1	1	15	4	1	1	1
Feeder	37	40	1	2	97	65	2	2	1.75
Global score			0.82				1.82		1.29

Discussion and Conclusions

In the scope of the recent Belgian *Salmonella* legislation (2), the use of hygienograms should be promoted. Evaluation of C&D protocols should be performed regularly in pig facilities to monitor the efficacy of protocols and products. C&D according to the prescribed protocol of CID Lines revealed a lower global score as compared to the farmers' protocol. The hygienogram scores clearly show that feeders and floors are the critical points in C&D of pig facilities. Good hygiene scores can be obtained in pig facilities working according to the proposed protocol, especially when foaming is applied. In conclusion, the hygienogram scoring system presented in this study is applicable in the field for the evaluation of C&D protocols in pig facilities.

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Royal Decree Belgium 27/04/2007

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