Mycoplasma suis infection in suckling pigs on a Belgian farm

Mycoplasma suis *infectie bij zuigende biggen op een Belgisch bedrijf*

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ABSTRACT

Mycoplasma suis **(formerly known as** *Eperythrozoon suis***) is an epicellular bacterium that affects porcine red blood cells.** *M. suis* **infections occur worldwide and are associated with weakness and anemia in suckling and weaned pigs, and reproductive disorders in sows. The present field report describes the detection of** *M. suis* **in anemic piglets originating from a Belgian farrow-to-finish herd. The herd was experiencing increased piglet mortality (16%) in the farrowing unit and had a high percentage of repeat breeders (22%). A control program using antimicrobials and hygienic and sanitary measures significantly decreased the number of clinically anemic piglets and the mortality rate in the farrowing unit. However, it did not have any significant influence on the reproductive failure of the farm. The lack of a significant effect on reproductive failure was probably due to the circulation of porcine reproductive and respiratory syndrome virus (PRRSV) on the farm.**

SAMENVATTING

Mycoplasma suis (voorheen *Eperythrozoon suis* genaamd) is een epicellulaire bacterie die de rode bloedcellen bij varkens aantast. Infecties met deze kiem komen wereldwijd voor en worden gekenmerkt door zwakte en anemie bij zuigende en gespeende biggen en door reproductiestoornissen bij zeugen. In deze studie wordt de diagnose van *M. suis* bij anemische biggen op een Belgisch varkensbedrijf besproken. Het bedrijf kampte met een toegenomen biggensterfte in het kraamhok (16%) en een hoog percentage terugkomers (22%). Een controleprogramma waarbij antimicrobiële middelen evenals hygiënische en sanitaire maatregelen werden toegepast, deed het aantal klinisch anemische biggen en het sterftepercentage in het kraamhok significant dalen, maar had geen significante invloed op de reproductiestoornissen bij de zeugen. Dit zou verklaard kunnen worden door de circulatie van het porciene reproductief en respiratoir syndroom virus (PRRSV) bij de zeugen.

INTRODUCTION

Mycoplasma suis is an epicellular rod-shaped bacterium that attaches to – and causes deformity and damage to – the red blood cells of pigs (Zachary and Basgall, 1985). *M. suis* was previously classified as a rickettsia on the basis of biological and morphological characteristics. However, *M. suis* is now known to be a member of the order *Mycoplasmatales* based on molecular relatedness and phenotypic characteristics (Rikihisa *et al.*, 1997; Neimark *et al.*, 2001).

M. suis can be transmitted by biting arthropods, by transplacental infection, or by the transfer of contaminated blood (Smith, 1986). On the farm, morbidity can range from 10% up to 60% (Heinritzi, 1999). The clinical symptoms are characterized by anemia and weakness in newborn and feeder pigs and by poor growth rates, which delay slaughter in feeder pigs (Smith, 1986). The mortality rate due to a *M. suis* infection can range between 10 and 20%, but on some occasions has been known to reach up to 90% (Heinritzi, 1999). The infection in sows has been associated mostly with chronic anemia and reproductive failure (anestrous, delayed estrus, early embryonic death and abortion) (Holter and Andrews 1979; Brownback, 1981). Acute infection in sows

often occurs in the peri-parturient period (Henry 1979, Smith 1992) and is characterized by pyrexia, depression, decreased milk production and poor maternal behavior (Henry, 1979).

As a differential diagnosis for *M. suis*, several causes of anemia should be included, such as neonatal iso-erythrolysis, neonatal anemia due to navel cord bleeding, primary or secondary immune mediated hemolytic anemia, lead, selenium and copper intoxication, gastric ulcers and leptospirosis.

M. suis infection is best diagnosed by the demonstration of the DNA of the agent using hybridization (Oberst *et al.*, 1990; Messick *et al.*, 2000) or the polymerase chain reaction (PCR) (Gwaltney and Oberst, 1994; Messick *et al.*, 1999). The use of in-situ hybridization (ISH) has been investigated and may be valuable for studying the pathogenesis of a *M. suis* infection. *In-situ* hybridization has the advantage that it illustrates cellular detail and histological architecture, so that both lesions and small numbers of *M. suis* infected red blood cells can be studied in the same section (Ha *et al.*, 2005). Serodiagnostic tests, such as the complement fixation test, the indirect hemagglutination assay and the enzyme-linked immunosorbent assay (ELISA) for *M. suis* infections in pigs, are known to be inaccurate (Hoelzle *et al.*, 2006).

M. suis infections can be treated with antimicrobials such as tetracyclines. However, neither parenteral nor oral treatment for longer periods is able to eliminate the organism (Henderson *et al.*, 1997). Taking the transmission paths into account, the preventive measures should be based on biosecurity. This includes purchase policy, adequate control measures for ectoparasites and sanitary measures.

In the present report, the detection of a *M. suis* infection in a Belgian sow herd and the herd's response to a control program are described.

MATERIALS AND METHODS

Study population and general management practices

The herd, situated in East Flanders, contained 250 sows and 800 feeder pigs (York G.L., York*G.L.) and practiced a one-week production system with individual housing of sows.

The sows were moved to the farrowing unit 5 days before the expected farrowing date. At that moment they were washed with Sarnacuran® (foxim) and were in-feed medicated with potentiated sulphonamides until 5 days after farrowing (as a preventive measure against postpartum dysgalactia syndrome). They were vaccinated against enteric colibacillosis, atrophic rhinitis, *Aujeszky's* disease, parvovirosis and swine erysipelas.

The piglets received an iron injection on day 1 and day 3 and were castrated within the first three days after birth. They were vaccinated twice against *Mycoplasma hyopneumoniae*, the first time on day 3 and the second time on day 26 of age. The age at weaning was 28 days.

Clinical symptoms in the herd

The farm was affected by anemia (15% of the suckling piglets) and increased mortality in piglets. The anemia was present at birth and was related to the litter. Sixteen percent of the litters and several animals per litter (33%) were affected. These piglets were weak and pale, and many of them (10-20%) had fever (> 40.5°C). The overall mortality rate in suckling piglets was 16%. In the nursery, pigs with blue ears (possible indication for PRRSV) could occasionally be seen. In the sow unit, a higher percentage of repeat breeders (22%) was observed: 70% of these sows showed regular return to estrus, 30% were irregular repeat breeders.

Symptoms of anemia and increased mortality of suckling piglets were first seen in January 2002, 3 months after the purchase of the gilts (Yorkshire, United Kingdom). It was unknown if there were any symptoms of anemia in the original herd of the Yorkshire gilts. At the end of that year, porcine reproductive and respiratory syndrome virus (PRRSV) vaccination with a live vaccine (PorcilisÒ PRRS, Intervet) of sows was initiated by the herd veterinarian after PRRSV virus isolation from 3 weak-born piglets. This decreased the abortion percentage (from 11% to 4%) and the early farrowing rate percentage (from 9% to <2%), but not the number of pale and weak-born piglets, nor the percentage of repeat breeders.

Diagnostics

In March 2005, the Department of Obstetrics, Reproduction and Herd Health of the Faculty of Veterinary Medicine, Ghent University was consulted for veterinary advice. Blood samples from 4 one-week-old diseased suckling pigs were randomly collected on EDTA (1.6 mg EDTA/7 ml). A hematological profile consisting of a total erythrocyte and leucocyte count, hematocrit values, hemoglobin levels and differential leukocyte counts was performed, together with an acridin orange staining of a blood smear and a PCR test for *M. suis* and PRRSV on DNA extracted from whole blood (Hoelzle *et al.*, 2003). Twelve randomly selected sows (6 healthy sows and 6 that had aborted) and fifty non-anemic piglets at the age of 10 and 14 weeks were blood sampled and the sera were tested for antibodies against PRRSV (Yoon *et al.*, 1995; Nodelijk *et al.*, 1996). The sera of the sows were also examined for the presence of antibodies against *Leptospira* Pomona, *Leptospira* Tarassovi, *Leptospira* Bratislava, *Leptospira* Icterohemorrhagiae, *Leptospira* Grippotyphosa, *Leptospira* Hardjo and *Leptospira* Sejroe using microscopic agglutination tests (Alexander, 1986).

Control program

We advised the farmer to treat all sows at 6 weeks before farrowing and all neonates at day 1 parenterally with oxytetracycline, and to medicate the sows 3 times a year with oxytetracycline in the feed (600 ppm) during 7 consecutive days. The control measures for ectoparasites and sanitary measures concerning the use of needles and surgical equipment were applied more strictly (change needle for every litter, use two sets of surgical instruments so that the contaminated set can be placed in a properly diluted disinfectant and used for alternate litters, etc.). Good management (prevention of other diseases, reduction of stress factors), combined with the detection of carrier animals (using PCR) and culling of the positive sows was also advised.

Statistical analysis

The clinical parameters before and after the implementation of the control program were evaluated using Chi-square analysis, or Fisher's exact tests when small numbers were involved.

RESULTS

The hematological profile of the 4 diseased suckling piglets showed on average a low hemoglobin concentration (3.7 ±0.6 g/dl), a low hematocrit (11.7 ± 2.2%) and also a low red blood cell count (2.6 ± 0.5*10^6 /mm^3) with the presence of some reticulocytes. The average total bilirubin concentration was increased (1.6 ± 0.5 mg/dl) (Table 1). No deviant values were observed concerning the leukocyte and the differential leukocyte counts.

M. suis infection in the piglets was confirmed by microscopy of acridin orange-stained blood smears in 2 out of 4 samples. The PCR tests were positive in the 2 samples that were also positive in the acridin orange-stained blood smears. The PCR tests for PRRSV were negative in all 4 samples. The results of the blood samples taken from 50 non-anemic piglets showed a seroconversion against PRRSV in 80 % of the piglets (40/50) at the end of the nursery.

In none of the sera of the 12 sows were antibodies to any *Leptospira* serotype detected, but high sample-to-positive (S/P) ratios were found for PRRSV (on average 1.8).

Three months after the implementation of the control program, the number of clinically anemic piglets and the mortality rate in the suckling piglets were significantly decreased (Table 2). The percentage of repeat breeders was not significantly decreased.

DISCUSSION

The present report describes a herd problem of increased mortality in suckling piglets due to a *M. suis* infection in Belgium. The most remarkable symptoms

in this herd were the increased piglet mortality in the farrowing unit and the high percentage of anemic piglets. The percentage of repeat breeders was also increased. These clinical symptoms can be indicative, but not pathognomonic, for a *M. suis* infection.

The hematological profile of the 4 diseased suckling piglets (Table 1) showed a decrease of the hemoglobin, a low hematocrit and also a decrease of red blood cells with the presence of reticulocytes indicating a regenerative anemia. The increase of the total bilirubin concentration is typical for hemolytic anemia. In the acute form of hemolytic anemia, an increase in the amount of free (indirect) bilirubin can occur in the blood, as conjugation to glucuronic acid in the liver is depressed (Heinritzi, 1999).

Considering the differential diagnosis of anemia, we could exclude neonatal iso-erythrolysis, immune mediated regenerative hemolytic anemia, hemolysis due to a drug-induced hypersensitivity reaction (*e.g.* to penicillins, potentiated sulphonamides or vaccines), lead and selenium intoxication and leptospirosis. Infections with *Leptospira* species may indeed be characterized by hemolysis, but anemia as the only clinical symptom is seldom observed (Decker *et al.*,

Table 1. Hematological profile of 4 diseased piglets.

	Results (average ± SD[a])	Reference values[b]
Hemoglobin concentration (g/dl)	3.7 ± 0.6	9 - 14
Hematocrit (vol%)	11.7 ± 2.2	26 - 41
Red blood cell count (10^6/mm^3)	2.6 ± 0.5	5 - 8
Total bilirubin concentration (mg/dl)	1.6 ± 0.5	0.05 - 0.29

[a] SD = Standard deviation
[b] Source: Friendship *et al.* (1984)

Table 2. Clinical parameters before and after the implementation of the control program to control *M. suis* infection.

	3 months before control program	3 months after control program
% of anemic piglets	15.0[a]	3.6[b]
% of mortality in the farrowing unit	16.0[a]	9.0[b]
% of repeat breeders (regular and irregular)	22.0[a]	17.0[a]

[ab] Within a row, data with a different superscript are significantly different (p< 0.05)

1970) and antibodies to *Leptospira* serotypes were not detected in the sera of the sows. Therefore, it is unlikely that leptospirosis was the cause of the anemia on this farm. The clinical observations, the detection of *M. suis* organisms in the blood smears, the positive PCR tests for *M. suis* and the negative PCR tests for PRRSV strongly suggest that infection with *M. suis* had caused the clinical problems of anemia in this herd.

We advised the implementation of a control program based on the application of antimicrobials, good management and measures to avoid the transmission of *M. suis* between animals, including control measures for ectoparasites and sanitary measures relating to the use of needles and surgical equipment. Transmission can indeed occur through biting arthropods and through contaminated blood (Smith, 1986). Furthermore, we advised the owner to detect carrier animals and to use this as a selection criteria for the culling of sows.

Three months after the implementation of the control program, the number of clinically anemic piglets and the mortality rate in the suckling pigs were significantly decreased. The percentage of repeat breeders, however, was not significantly decreased. *M. suis* infection can cause reproductive disorders (Brownback, 1981), but in this case, based on the results of the serological tests, an endemic PRRSV infection was likely the cause of the higher percentage of repeat breeders. Due to the fact that PRRSV infection causes immunosuppression, it is a predisposing factor in the development of many diseases, including *M. suis* infections (Gresham *et al.*, 1994).

In conclusion, a *M. suis* infection was diagnosed as the main cause of the clinical symptoms in the suckling piglets. A *M. suis* infection should be included in the differential diagnosis for every farm suffering from problems with weak, pale piglets, a higher mortality rate in suckling piglets, and reproductive disorders. The measures taken were sufficient to control the *M. suis* infection on the farm. Since the long-term use of antimicrobials cannot be considered a durable solution for the problem, we advised the implementation of a more strict control program, including the detection of carrier animals (using PCR) and the culling of positive sows.

ACKNOWLEDGMENTS

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