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Monitoring of *Lawsonia intracellularis* in breeding herd gilts

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ABSTRACT

In modern pig production, proliferative enteropathy is a common cause of diarrhoea and poor growth in young animals. This study aimed to determine the possible spread of *Lawsonia intracellularis* through the sale of replacement gilts and the possibility to protect the herds by adequate biosecurity measures. This was achieved by repeated sampling of 50 gilts in an infected multiplying herd, from the last day in the farrowing pen and until sale. Further, 60 gilts sold from this herd were tested during their stay in quarantine in a recipient herd. To confirm freedom from infection, 100 growing pigs in the recipient herd were also tested. Individual faecal ($n = 748$) and blood ($n = 728$) samples were analysed by PCR and ELISA, respectively. Transmission of *L. intracellularis* from the sows to their offspring was not demonstrated. However, the possible transmission between herds by replacement gilts was demonstrated. Peak shedding occurred at 12 and 15 weeks of age, and single animals were also PCR-positive at 24–36 weeks of age in the multiplying herd and in the quarantine in the recipient herd. Further, the possible occurrence of chronically infected carrier animals was suggested. Although *L. intracellularis* is widely spread, it appears possible to avoid the transmission between herds by employing adequate biosecurity measures. Thus, it would be advisable to establish herd profiles in breeding herds to avoid the selling of infected animals as well as to establish the health status of the recipient herd. Further, the health status of the recipient herds should be known.

1. Introduction

Proliferative enteropathy, manifested as diarrhoea and poor performance in growing pigs, or as acute haemorrhagic enteropathy with high mortalities in gilts and finisher pigs, is widespread in pig production (Guedes et al., 2002b; Jacobson et al., 2003b). The causative organism, *Lawsonia intracellularis*, has been demonstrated globally with herd prevalences ranging from 48 to 100% (Lee et al., 2001; Jacobson et al., 2005). The microbe was first described in 1993 (Gebhart et al., 1993) and still, features of the disease such as the transmission within and between herds, remain to be fully elucidated (Lawson and Gebhart, 2000; McOrist et al., 2003). The microbe may be viable for 14 days outside the host (Collins et al., 2000) and rodents are thought to only play a minor role in the spread (McOrist et al., 2003). Thus, pig-to-pig transmission is considered as the main route of infection (Jordan et al., 2004) and the possibility that single gilts transmit the microbe to their offspring has occasionally been suggested (Jensen et al., 2005; Kroll et al., 2005). A few infected piglets will carry the bacterium to the grower-finisher facilities were the main shedding and spread will occur (Bronsvoort et al., 2001; Jensen et al., 2005). Within these

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units, the infection may persist due to poor sanitation measures and short empty-time space between batches (Smith et al., 1998; McOrist et al., 1999). Experimentally, shedding has been detected 1–12 weeks post-inoculation, whereas antibodies have been detected in sera from 2 to 13 weeks (Smith and McOrist, 1997; Guedes and Gebhart, 2003). However, only a few studies have addressed the sequential shedding (Guedes et al., 2002b; Stege et al., 2004; Jensen et al., 2005) or seroconversion in conventional herds (Just et al., 2001; Marsteller et al., 2003). In these, peak shedding occurred between 10 and 16 weeks of age and persisted for 2–6 weeks. Seroconversion was seen from 10 to 24 weeks of age, although serum antibodies only persisted for 1–6 weeks in single animals. In the spread between herds, the replacement animals are considered to be of major importance (Smith et al., 1998; McOrist et al., 2003). However, the actual infection status of breeding animals close to the introduction into new herds has rarely been investigated (Friendship et al., 2005).

Hence, the aim of this study was to determine the possible spread of *L. intracellularis* through the purchase of infected breeding herd gilts. Shedding and the development of circulatory antibodies were determined during rearing and at the time of transfer from a breeding herd selling gilts at various ages. Further, the possibility to protect the recipient herd from the infection by adequate biosecurity measures was investigated by repeated sampling of gilts sold from the breeding herd during their stay in quarantine in a recipient herd.

2. Materials and methods

The study was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

2.1. Herds

Pigs from the breeding herd had tested positive for *L. intracellularis* by necropsy and by PCR on faecal samples 3 months prior to the start of the study. Batches of 18 sows farrowed at 4 weeks interval. Piglets were weaned at 5 weeks of age and remained in the farrowing pen for 1 additional week before they were transferred to the grower facilities in a separate building. Between each batch, all units were washed, disinfected and left empty for 1 week. Litters were not mixed. The pigs remained in the growing unit for 4–6 weeks and thereafter, gilts were moved to an un-insulated barn and mixed in two large pens with deep straw litter bedding. Within this unit, gilts were moved and mixed 3–4 times. On average, 10–15% of the gilts were sold as pre-pubertal gilts (*i.e.* ~12–14 weeks old), 10–15% were sold as young gilts (~22 weeks of age), 10–15% as gilts for mating (~30 weeks of age) and 55–70% as pregnant gilts (~42 weeks of age).

The recipient herd was a 160-sow, piglet-producing herd where *L. intracellularis* had never been demonstrated (Jacobson et al., 2003b). Usually, six young gilts and six gilts for mating were bought every second month from the multiplying herd and introduced into the quarantine, located at a 1-km distance from the main herd. Separate equipment, clothing and footwear were used. The gilts remained here until they were ready for mating (3–9 weeks). In the main herd, the sows farrowed in batches of 30 sows into nursery units that had previously been washed with hot water under high pressure followed by disinfection with Virkon S (Pharmacia & Upjohn Animal Health AB, Helsingborg, Sweden). Piglets were weaned at 5 weeks of age and remained in the nursery for 1–2 additional weeks, before they were moved to the grower facilities, mixed and sorted according to weight. The piglets were sold at 75 days of age (~30 kg b. w.). The post-weaning mortality was 0.4–1.5% and the average piglet growth rate was 500 g/day.

2.2. Animals and experimental design

In the breeding herd, 50 individually ear-tagged, crossbred Yorkshire × Swedish Landrace gilts (1–3 per litter), born within 1 week and in one farrowing unit, were included in the study. Faecal and blood samples were collected every 3rd week from the last day in the farrowing pen (*i.e.* 1 week post-weaning) and until sale. The pigs were sold to eight different herds.

The recipient herd only bought gilts from the breeding herd. All of the 60 gilts (22–35 weeks old) bought during 1 year were included in the study. Sampling was performed at arrival, after 3 weeks stay in quarantine, on one occasion also after 6 weeks stay in quarantine, and before the gilts were transferred to the main herd. To further confirm the infection status of the herd, samples were obtained from 20 growing pigs in the age of 10–12 weeks on five occasions during a 1-year period (in total 100 pigs). With one exception, individual rectal faecal samples and blood samples were collected at each occasion.

2.3. Analysis

Faecal samples were stored at −80 °C until analysis by nested PCR as previously described (Jacobson et al., 2004). All reactions were performed in 25-μL reaction volume and 1 μL of an internal control (mimic) was added to each tube (Jacobson et al., 2003a). Positive and negative controls were included in each run. Inhibited samples were re-analysed by PCR once or twice, and if results were still not obtained, faecal DNA was extracted by a commercial kit (QIAamp® DNA Stool Mini Kit, Qiagen Inc., Valencia, CA, USA) and analysed by conventional PCR (Jacobson et al., 2004).

Blood samples without additives were centrifuged and sera were stored at −20 °C until analysis by blocking ELISA according to the instructions given by the manufacturer (Svanova Biotech, Uppsala, Sweden, and Bioscreen GmbH, Münster, Germany). Validation of this method has not been published in a scientifically scrutinised paper, but the stated sensitivity was 96.5% and the specificity, 98.7% (Svanova biotech, unpublished results). The resulting OD value was read at 450 nm and converted to the calculated percent inhibition (PI). Values above PI 35 were regarded as positive.