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SEROPREVALENCE OF *LAWSONIA INTRACELLULARIS* IN LARGE PIG PRODUCTION UNITS

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In 11 'farrow-to-finish' outdoor or indoor production units, blood samples from late pregnant gilts were tested by indirect immunofluorescence antibody (IFA) serum assay for *Lawsonia intracellularis*. The offspring of positively tested gilts were tested at 2, 7, 12, 17, 22 and 27 weeks of age for seroprevalence of *Lawsonia intracellularis*. All offspring of IFA positive gilts were seronegative at 2 and 7 weeks of age. At 12 weeks of age 81.0% of indoor and 51.0% of outdoor pigs were tested positive. While at 17 weeks of age 82.5% of indoor-raised pigs showed seropositivity, in outdoor units the seropositivity declined to 31.3%. At weeks 22 and 27 indoor-raised pigs still showed marked seropositivity (17.7% and 11.5%) but their outdoor-raised counterparts revealed declining values (7.4% and 0%).

Key words: Pig, *Lawsonia intracellularis,* seroprevalence, outdoor

Porcine proliferative enteropathy (PPE) is a common enteric disease of growing and finishing pigs (Dufresne, 1998). The aetiologic agent has been identified as *Lawsonia intracellularis* (LI) (McOrist et al., 1995). Despite the broad range of animals in which LI infection has been detected, LI is most significant in pigs (Dufresne, 1998). Although specific detection of LI is exacting, incidence of infection in pigs has been shown to be high in numerous surveys and the disease accounts for significant economic losses (Lanza et al., 1996; Bane et al., 1997; Bronsworth et al., 2001).

The infection is transmitted by oral-faecal route and the infective dose is very low (Lanza et al., 1996). It seems to be likely that transmission of the infection may occur from the sow to the piglets during the first days of life, since sucking piglets have been shown to shed the organism (Bronsworth et al., 2001). Especially gilts are potential carriers of the disease and infect their piglets in early life (Lanza et al., 1996). The incubation period is approximately 2 weeks and even subclinically infected animals may shed the organism (Dufresne, 1998).

During infection, LI attaches to and invades the intestinal epithelial cells (Lanza et al., 1996). Following entry, bacterial cells must escape the entry vacuole and initiate heavy intracytoplasmic growth (McOrist et al., 1995). As a consequence of infection, alterations in cell function and modulation of immune re-

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sponse occur (Bronsworth et al., 2001). Analysis of the steps in LI infection improves the understanding of virulence determinants, host responses, susceptibility and resistance mechanisms which are important factors in prevention, treatment and diagnosis (Bane et al., 1997). The disease spectrum varies from sub-acute enteritis to fatal enterocolitis (Dufresne, 1998). The disease has two clinical forms, namely Porcine Intestinal Adenomatosis (PIA), currently seen in growing pigs, and Proliferative Haemorrhagic Enteropathy (PHE) in fattening pigs (Dufresne, 1998). The disease causes significant economic losses worldwide (McOrist et al., 1995). PPE has been identified with increasing frequency as a cause of diarrhoea and poor growth during the growing-finishing period (McOrist et al., 1995). It may manifest itself in poor growth rate, (bloody) diarrhoea, stunting or sudden death in late finishing pigs or replacement breeding animals (Dufresne, 1998). PPE occurs virtually in all swine production systems (Lanza et al., 1996). A recent serologic survey found 96% seroprevalence of LI in US herds (Bane et al., 1997). Under field conditions, identifying the stage of LI infection allows producers to more effectively target their antimicrobial prevention and treatment regiments (Dufresne, 1998).

Epidemiological factors responsible for variation in the prevalence and severity of LI infection under field conditions are incompletely understood (Just et al., 2001). Under field conditions, various farm management factors may influence the development, severity and therapeutic response to PPE. Movement of pigs, nutritional changes, feed antibiotic usage, temperature fluctuations, pig density, pig age, facility design, sanitation, immune status, resistance, genetic susceptibility and outdoor raising have been proposed as factors influencing the clinicopathological expression of PPE outbreaks in endemically infected herds (Bane et al., 1997).

Central European surveys on the seroprevalence of LI in large pig production units are still lacking.

Materials and methods

The study was performed in 11 'farrow-to-finish' production units, belonging to the same commercial swine production system in southern Germany. The farms had previously experienced clinical PPE (diarrhoea, stunting, dullness, apathy, unthriftiness in growing pigs and sudden death or bloody diarrhoea in late finishing pigs and replacement breeding animals). At the time of the study, intermittent diarrhoea and poor growing-finishing growth rates and deterioration of feed efficiency were the only clinical signs of a possible PPE infection. Especially pigs of 6–16 weeks of age showed reduction in growth rates and stunting.

All units had indoor farrowing and nursery facilities. Five of these units produced growing-finishing pigs in large indoor barns, 6 of them in outdoor huts.

The piglets were uniformly weaned at days 21–24 and moved to the nursery 'flat-deck' barns. These animals were moved again at a weight of 21 ± 2.7 kg (age of 7–8 weeks) to the growing/finishing area, and were kept either in barns

(indoor) or open huts (outdoor). No prophylactic antimicrobial was used. Pigs raised indoors were kept in large pens (15–20 pigs per pen = 0.8–0.9 m^2/pig) on 1/3 slatted floors and were fed *ad libitum* with identical commercial diet. Outdoor-raised pigs were kept in groups of 15–18 in large open huts with deep straw bedding on large pasture of 20–50 m^2 per pig and received the same diet as their indoor-raised counterparts.

Before the trial the seroprevalence for LI was tested. Blood samples from late pregnant gilts (10% of female inventory) were collected in each unit and an indirect immunofluorescence antibody (IFA) serum assay was used to detect anti-LI IgG antibodies as described by Knittel (1997). IFA testing was performed in the Minnesota Diagnostic Laboratory. IFA has proven to be a sensitive and specific test (Schwartz et al., 1999). The coating antigen was a pure culture of LI strain N343. An anti-porcine IgG-fluorescein-isothiocyanate conjugate (diluted 1:30) was bound to porcine IgG (diluted 1:30 in phosphate buffered saline, PBS) that was bound to LI-infected cell cultures in the wells of 72-well microtitration plates. Plates were examined by fluorescein microscopy, and wells with fluorescing bacteria were interpreted as positive.

After the seropositivity of the pregnant gilts has been determined, one newborn piglet from each positively tested gilt litter was identified by ear tags and repeatedly tested until slaughter for seroprevalence of LI. Blood samples were collected from the same animals at 2, 7, 12, 17, 22 and 27 weeks of age.

Results

Seroprevalence of LI ranged from 50–94.7% of the pregnant gilts in the units tested (Table 1). All offspring of IFA-positive gilts were seronegative at 2 (still in farrowing cages) and 7 (last week of nursery) weeks of age (Table 2). Testing at 12 weeks of age revealed positive IFA results in 81.0% of indoor and 51.0% of outdoor pigs. While at 17 weeks of age 82.5% of indoor-raised pigs showed seropositivity, the seropositivity declined in outdoor units to 31.3%. Indoor-raised pigs still showed seropositivity at weeks 22 (17.7%) and 27 (11.5%), but their outdoor-raised counterparts revealed declining values (7.4% and 0%) (Table 2).

Discussion

Environmental factors, including the design of the facility, management strategies, general hygiene, population density and feed ingredients affect the prevalence and severity of PPE (Bane et al., 1997). Information is still lacking on the persistence of the bacteria in the environment, the presence of animal vectors and other risk factors associated with PPE (Lanza et al., 1996).

Table 1

Seroprevalence of *Lawsonia intracellularis* in 11 large pig production units

Unit, number of inventoried females			Number of animals tested	Number/percent of positive animals
1	indoor	111	11	9/81.8
2	indoor	156	16	13/81.3
3	indoor	132	13	10/76.9
4	indoor	98	10	6/60.0
5	indoor	189	19	11/57.9
6	outdoor	151	15	11/73.3
7	outdoor	188	19	18/94.7
8	outdoor	201	20	13/65.0
9	outdoor	302	30	24/80.0
10	outdoor	121	12	6/50.0
11	outdoor	179	18	12/66.7

Table 2

IFA-positive sera in pigs born to *Lawsonia intracellularis* IFA-positive gilts in indoor or outdoor production systems

Age of pigs in weeks	Number and percent of positive sera					
	2	7	12	17	22	27
Indoor	69/0	68/0	63/51(81.0)	63/52(82.5)	62/11(17.7)	62/7(11.5)
Outdoor	114/0	101/0	100/51(51.0)	96/30(31.3)	95/7(7.4)	95/0

Lanza et al. (1996) and Smith and McOrist (1997) have shown a high prevalence of PPE infections on all types of farms, regardless of the management system. The subclinical form of the disease is by far the most common manifestation of PPE (Dufresne, 1998). In endemically infected herds, clinical manifestation seems to be precipitated by stress and commingling (Bane et al., 1997). The best way of preventing the disease is by maintaining the unit free from the infection (Bronsworth et al., 2001). New breeding stock should be purchased only from herds known to be free from the infection (Bronsworth et al., 2001).

Historically, PPE has been diagnosed by postmortem examination and histopathology (Just et al., 2001). More recently, polymerase chain reaction (PCR) tests have been developed to detect LI in faeces and tissues (Jones et al., 1993). Indirect fluorescein antibody (IFA) test has been developed to detect IgG antibodies specific for LI (Knittel et al., 1998). In the present trial IFA assay, instead of PCR, was used to determine the prevalence and timing of exposure to LI because it was likely that PCR would detect only pigs with clinical signs, or those shedding the organism. Although PCR is a highly sensitive test, only ani-

mals with active lesions excrete the organism in numbers sufficient for detection by PCR (Jensen et al., 1997). Shedding LI may be cyclical, even in animals where the ileum is colonised (Knittel et al., 1997). Therefore a test that detects the immune response to LI is more accurate for diagnosing ileitis than a test that attempts to detect the organism in either the faeces or the tissues (Guedes et al., 1999). However, as with all serological tests, IFA identifies previous exposure, not active infection. According to Knittel et al. (1998) the sensitivity of IFA was estimated to be 0.90 and the specificity was estimated to be 0.99.

The negative test results at 2 and 7 weeks of age cast doubt on the role of passively acquired antibodies of young pigs [or the ability of the IFA tests to detect passively acquired antibodies (Lawson et al., 1998)]. In the present trial seroprevalence decreased with age. It appears, therefore, that the time of exposure to seroconversion and back to the seronegative status may be as short as a few weeks. It is also possible that more animals have been infected, but IFA was unable to detect them because of the short duration of seropositivity. According to Knittel et al. (1998) LI is transmitted via the faeces, and seroconversion (and possible clinical signs) occur 2–3 weeks after exposure. As none of the animals were tested positive until week 12 (grower-finisher facility), it appears that exposure occurred during the last weeks in the nursery and the first 2 weeks of the growing-finishing phase. In the present trial exposure from the sow (possibly shedding the organism) either did not occur or was undetected. In a similar trial (Just et al., 2001) 15% of the tested animals seroconverted after infection and became seronegative five weeks later. Consistently with our findings, other authors (Just et al., 2001) concluded that seroconversion to LI occurred after the pigs had entered the growing-finishing site, suggesting that exposure had taken place in the nursery.

If ileitis is diagnosed, there are no successful methods of eradicating the infection from a herd (Lanza et al., 1996). Nevertheless, there are practical methods for 'living with the disease', minimising economical losses and preventing clinical outbreaks (Dufresne, 1998). Since LI is an obligate intracellular organism that infects only epithelial cells, the antimicrobial of choice has to be able to concentrate inside the cell and stop bacterial multiplication (Dufresne, 1998). As treatment is often unrewarding and economically questionable, prevention and control measures should be implemented. The most effective means of control is to prevent the development of PPE lesions by administering feed-grade antibiotics. Tylosin, chlortetracycline, lincomycin, spectinomycin, bacitracin, tiamulin (Just et al., 2001) and oregano ethereal oils (Sivropoulou et al., 1996; Tsinas et al., 1998) are effective in the prophylaxis of LI infections. According to Sick et al. (2002) Enterisol® Ileitis ALC vaccine (USDA, USA) is a safe, labour-saving (due to oral application) and efficient method for the prevention and control of PPE in the US. Enterisol® is not registered in Europe. The present results suggest that in large outdoor environment a lower LI infectious pressure results in lower (re-)infection rate and a faster decline of seropositivity.

References

Bane, D., Norby, B., Gardner, I., Roof, M., Knittel, J. and Bush, E. (1997): Prevalence of management risk factors associated with *Lawsonia intracellularis* seropositivity in the U.S. swine herd. Proc. Leman Swine Conf. Suppl. pp. 10–11.

Bronsworth, M., Norby, B., Bane, D. P. and Gardner, I. A. (2001): Management factors associated with seropositivity to *Lawsonia intracellularis* in US herds. Swine Health and Production **9,** 285–290.

Dufresne, L. (1998): Alimentary tract disorders of growing pigs. Proc. 15th IPVS Congr., Birmingham. pp. 71–85.

Guedes, R., Gebhart, C., Winkelman, N. and Marsteller, T. (1999): Comparison of different methods for diagnosis of porcine proliferative enteropathy. Proc. Conf. of Res. Workers in An. Dis. p. 99.

Jensen, T., Moller, K., Leser, T. and Jorsal, S. (1997): Comparison of histology, immunohistochemistry and polymerase chain reaction for detection of *Lawsonia intracellularis* in natural porcine proliferative enteropathy. Eur. J. Vet. Pathol. **3,** 15–18.

Jones, G., Ward, G., Murtaugh, M., Lin, G. and Gebhard, C. (1993): Enhanced detection of the intracellular organism of swine proliferative enteritis ileal symbiont intracellularis in feces by polymerase chain reaction. J. Clin. Microbiol. **31,** 2611–2615.

Just, S. D., Thoen, C. O., Thaker, B. J. and Thompson, J. U. (2001): Monitoring of *Lawsonia intracellularis* by indirect serum immunofluorescence assay in a commercial swine production system. Swine Health and Production **9,** 57–61.

Knittel, J. P. (1997): Diagnosis of infection with *Lawsonia intracellularis* in swine. MSc Thesis, Iowa State University.

Knittel, J. P., Jordan, D., Schwartz, K., Janke, B., Roof, M., McOrist, S. and Harris, D. (1998): Evaluation of ante mortem polymerase chain reaction and serological methods for the detection of *Lawsonia intracellularis*. Am. J. Vet. Res. **526,** 722–726.

Knittel, J. P., Schwartz, K., McOrist, S., Roof, M., Jordan, D. and Harris, D. (1997): Diagnosis of porcine proliferative enteritis. Comp. Cont. Ed. Food Anim. **19,** 26–29.

Lanza, I., Pozo, J., Munoz, M., Rubio, P. and Carmenes, P. (1996): Epidemiological study of porcine proliferative enteropathy in Spain. Proc. 14th IPVS Congress, Bologna, Italy. p. 259.

Lawson, S., McOrist, S. and Rowland, A. (1998): Serological diagnosis of the porcine proliferative enteropathies: Implications for aetiology and epidemiology. Vet. Rec. **122,** 533–536.

McOrist, S., Jasni, S., Mackie, R., MacIntyre, N., Neef, N. and Lawson, G. (1995): Reproduction of porcine proliferative enteropathy with pure cultures of ileal symbiont intracellularis. Infect. Immun. **61,** 4286–4292.

Schwartz, K., Knittel, J., Walter, D., Roof, M. and Anderson, M. (1999): Effect of oral tiamulin on the development of porcine proliferative enteropathy in a pure culture challenge model. Swine Health and Production **7,** 5–11.

Sick, F., Hayes, P. and Kolb, J. (2002): Enterisol® Ileitis vaccine; Field efficacy evaluation. Proc. AASV, Minnesota. pp. 161–163.

Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T. and Arsenakis, M. (1996): Antimicrobial and cytotoxic activities of Origanum essential oils. J. Agr. Food Chem. **44,** 1202–1205.

Smith, S. and McOrist, S. (1997): Development of persistent intestinal infection and excretion of *Lawsonia intracellularis* by piglets. Res. Vet. Sci. **62,** 6–10.

Tsinas, A. C., Kyriakis, S. C., Bourtzi-Chatzopoulou, E., Arsenakis, M., Sarris, M., Papasteriades, A. and Lekkas, S. (1998): Control of porcine proliferative enteropathy by feed application of Origanum essential oils. Proc. 15th IPVS Congress, Birmingham, England, p. 220.