Sows Seropositive to *Lawsonia intracellularis* (LI) Influence Performance and LI Seropositivity of their Offspring

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Abstract

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The aim of the study was to test, if seropositivity to *L. intracellularis* does decline more rapidly in the offspring of *Lawsonia intracellularis* (LI) positive gilts than in the offspring of LI negative gilts in units infected with endemic Porcine Proliferative Enteropathy (PPE). In a Croatian pig-production unit late-pregnant gilts were tested by an indirect immunofluorescence antibody (IFA) serum assay for LI. Twenty IFA-positive and 20 IFA-negative gilts were selected. Their 2-week-old piglets (191 from positive gilts, and 192 from negative gilts) were inoculated intragastrically with pure-culture of LI. These animals were tested until 25 weeks of age for seroprevalence of LI. Blood samples were collected at 5, 10, 15, 20 and 25 weeks of age.

Seropositivity in offspring of IFA negative gilts was highest at the age of 5 weeks (85.9%) and declined gradually from week 10 to only 6.3% at week 25. At the same time, the offspring of IFA positive gilts showed lower and faster-decaying seroprevalence: 21.4% at week 5 and 0% at weeks 15, 20, and 25. There was no significant difference in the occurrence of diarrhoea between the offspring of LI seropositive and seronegative gilts. Mortality was significantly ($p < 0.05$) higher (20.4%) among offspring of seronegative, compared to 14.1% the offspring of seropositive gilts. Average weight gains were higher ($p < 0.05$, 741 ± 44g vs. 611 ± 41g·d^{-1}) among the offspring of seropositive than among of seronegative gilts. The essence of this report is the observation that seropositivity to LI declines more rapidly in grower/finisher pigs born to LI seropositive gilts than the ones born to LI seronegative gilts in units infected with endemic PPE.

Gilts, suckling piglets, seroprevalence, mortality, weight gain

Porcine proliferative enteropathies (PPE), caused by *Lawsonia intracellularis* (LI) are common infectious diseases that affect weaned and growing-finishing pigs of various ages (Jones et al. 1993). Baumann and Bilkei (2002) found in 29% of the postmortem-examined culled fattening growing pigs (n = 1319) LI infection. The infection has two types of clinical expression: porcine intestinal adenomatosis (PIA) in growing pigs and proliferative haemorrhagic enteropathy (PHE) in fattening pigs and replacement gilts (Dufresne 1998).

Various farm management factors such as 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 may influence the development and severity of PPE (Hagen and Bilkei 2003; Bona and Bilkei 2003). There is a biological basis to support the suggestion that positive LI sero-status of the sows is associated with lower LI titre of protective antibodies in their offspring as a result of lack of exposure and/or higher passive immunity to the organism (Winkelman 1996b). However, this association should be further investigated.

In the present trial, the hypothesis was that the offspring of indirect immunofluorescence antibody (IFA)-positive gilts would present lower and faster-decaying seroprevalence against LI after a pure-culture challenge, and would have better production parameter compared with the offspring of IFA-negative gilts.

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Materials and Methods

The study was performed in a 800-sow Croatian pig-production unit from February to October 2003. Pre-trial necropsies of culled animals revealed a low infectious pressure of LI and *Brachyspira hyodysenteriae.* The unit was free of *Salmonella choleraesuis* and verotoxigenic *Escherichia coli*.

The breeding females were F1 crosses of Large White and German Landrace breeds. Late-pregnancy gilts (n = 193) were tested for seroprevalence for LI by an IFA serum assay to detect anti-LI IgG antibodies (Knittel et al. 1997): an anti-porcine IgG-fluorescein-isothioncyanate conjugate, diluted in 1:10 PBS (phosphate-buffered saline) that was bound to porcine IgG (diluted in 1:30 PBS) that was bound to LI-infected cell cultures in the wells of 72-well microtitration plates. Plates were examined by fluorescent microscopy, and wells with fluorescing bacteria were interpreted as positive. Animals were classified as IFA-positive at dilution of ≥ 1:30. The IFA coating antigen was a pure culture of LI strain N343. Both pig serum and the anti-swine immunoglobulin were diluted 1:30 in PBS before testing. Samples that showed fluorescence were judged as positive.

After the seropositivity of the animals has been tested, 20 IFA-positive (1:30-120) and 20 IFA-negative gilts were selected by computer-generated list within blocks of similar age in a 1:1 assignment ratio. Before challenge, the suckling piglets' faeces (one in each litter) were tested for LI by modified Ziehl-Neelsen staining (Baumann and Bilkei 2002) 3 times (at days 7,10 and 14 *post partum*). Then, the 2-week-old piglets (191 from positive gilts, and 192 from the negative gilts) were inoculated intragastrically with pure culture of LI as follows: LI field isolate was subjected to low-passage culture techniques for 12 weeks, harvested and processed as described by McOrist et al. (1993). The piglets were inoculated with 106 organisms in a 20-ml inoculum by gastric intubation. Twenty mL of inoculum and 80mL of air were drawn into a 100 ml syringe and administered to each pig (the air flushing all inoculum into the stomach).

All pigs were tested at 5-week intervals until age of 25 weeks for seroprevalence for LI, analogously to the testing of the pregnant gilts. Animals were classified as IFA-positive at dilution of ≥1:30. Blood samples were collected at 5, 10, 15, 20, and 25 weeks of age. The litters were kept together until weaning.

The piglets were weaned at day 35 ± 2.1 d of age and moved to the nursery "flat-deck" barns. Animals born to IFA-positive and IFA-negative gilts were separated in the nursery, but received the same commercial diet *ad libitum.* The nursery animals were moved again at a weight of 22 ± 1.3 kg to the grower-fattener houses. The grower-fattener animals were separated, and raised under the same management and environment in large pens (17-20 pigs per pen = 0.96 m^2 / pig) and were fed *ad libitum* with an identical commercial diet. No prophylactic antimicrobial was used.

Mortality from challenge to slaughter and average daily weight gain (ADG), were both in nursery and in grower-fattener unit separately analysed. Dead piglets were diagnosed according to previous clinical signs as anorexia, dullness, apathy, wasting, moderate irregular diarrhoea with normal colour, reduction of weight gain. Necropsy revealed different manifestations of PIA and PHE lesions. IFA testing confirmed PPE. According to Hagen and Bilkei (2003) IFA has been proven to be a sensitive and specific test.

Diarrhoea in suckling piglet litters was recorded and compared between LI seropositive and seronegative gilt litters. Days, when more than 10% of the nursery and grower-fattening animals showed diarrhoea (semi-solid to watery stools) were recorded and compared between the offspring of the LI seropositive and seronegative gilts.

Serological data were analyzed by analysis of variance (Statistix, Analytical Software Inc. Tallahassee, Fl, USA). Mortality was defined as the percentage of death in a pen due to PPE. Diarrhoea data were recorded as "yes/no". Average daily gain was calculated by dividing total weight gain of the pen by the number of pig-days. All data presented are means ± standard deviation (SD). Differences in ADG between the offspring of LI-seropositive and LI-seronegative gilts were investigated using a pooled analysis of variance, weighted on the number of pigs per pen. The final weight variable were regressed on birth-weight and gender retaining variables with P-value of 0.05 for the multivariate model in a backward elimination process (SAS User's Guide: Statistics, Cary, North Carolina, SAS Inst. Inc. 1988).

Results

Pre-trial faecal screening revealed in 7 piglets of LI-seropositive sows the presence of LI; none of the piglets of LI-seronegative sows were faecal-positive for LI.

Seropositivity in offspring of IFA negative gilts was highest at age of 5 weeks (85.9%, 1:60 - 1:240) and declined gradually from week 10 to only 6.3% at week 25 (1:30 - 1:60). At the same time, the offspring of IFA positive gilts showed lower and faster-decaying seroprevalence: 21.4% at week 5 (1:60 – 1:120) and 0% at weeks 15, 20, and 25. There was no significant difference (9.9 ± 1.1 days vs. 10.4 ± 1.3 days) in the occurrence of diarrhoea between the offspring of LI seropositive and seronegative gilts either preweaning, or in the nursery (4.5 ± 0.4 days vs. 4.3 ± 0.3 days), or during the growing-fattening period (10.4 ± 0.6 days vs. 12.1 ± 1.1 days). Mortality was significantly ($p < 0.05$, 14.1% vs. 20.4%) lower in the offspring of seropositive compared to the offspring of seronegative females.

Nursery ADG differed significantly ($p = 0.04$) between to offspring of seropositive sows (391 ± 19 g·d^{-1}) and seronegative ones (322 ± 21 g·d^{-1}). Grower-fattener ADG showed a ($p = 0.03$) difference between the offspring of seropositive and seronegative females (741 ± 44 g vs. 611 ± 41 g·d^{-1}).

Discussion

Under conditions of this study, the offspring of LI seropositive sows were partly protected against LI when challenge exposed to LI. Our results indicate that LI seropositivity of the dams positively influences the course of seropositivity to LI and the health and weight gains of their offspring.

The pre-trial faecal screening revealed only in 7 piglets of LI seropositive sows the presence of LI but none of the piglets of LI seronegative sows were diagnosed positive for LI. Although seropositive sows might have excreted LI (and their offspring might have ingested it), maternal antibodies prevented LI infection and (except 7 piglets) excretion of LI before challenge. These findings are consistent with Philips and Geiger (1998) who found that under high pressure of natural occurring PPE, some of the pigs remained negative for LI by PCR and IFA until late nursery.

The present results suggests that the maternal derived passive immunity gave partial protection to the offspring of LI seropositive gilts against the long-term development of PPE and reinfections with LI. The longer seropositivity of the offspring of LI seronegative gilts suggests reinfections. LI can be present for weeks or months in proliferating crypt cells but without reinfections, faecal shedding and seropositivity can cease 4 weeks post challenge (Just et al. 2001).

In the present trial, IFA (instead of PCR) was used because it was likely that PCR would detect only clinically infected pigs or those shedding the organism. Shedding LI might be cyclical, even in animals where the ileum is colonized (Winkelman 1996a). Therefore, a test that detects an immune response to LI is more accurate (except for young suckling piglets with time-limited passive maternal antibody [Winkelman 1996a]) for diagnosing infection than a test that attempts to detect the organism either in faeces or tissue (Guedes et al. 1999).

Much of the information we have on humoral immune response of pigs were obtained through challenge trials using mucosal homogenates from pigs affected by PPE (Winkelman 1996b). Seroconversion in growing pigs during a natural outbreak of PIA showed that LI titers of 1:30 decayed within 3-4 weeks (Winkelman 1996a). Effective length of protection provided by passive immunity has not yet been established (Gebhart and Guedes 2001). The common occurrence of the disease and the wide age range of susceptible pigs suggests that natural immunity in field situations does not occur regularly (Jensen et al. 1997). According to Hagen and Bilkei (1993) in units with endemic PPE, outdoor raised growing-finishing pigs born to seropositive dams, reveal lower and nearly three times faster decaying seroprevalence to *L. intracellularis*, compared to indoor raised counterparts in the same unit.

According to Gebhart and Guedes (2001), sows that had survived PHE had IgG titers to LI at farrowing (suggesting that these sows confer passive immunity to their piglets). Humoral immune response of LI-infected pigs is weak (Gebhart and Guedes 2001). In a trial (Winkelman 1996b), IgG titers of 1:30 to LI appeared 2 weeks after challenge and up to 90% of the pigs became positive 3 weeks later, with 5% showing titers of ≥1:480. At 4 weeks post challenge, titers began to decay and a decreased percentage of the pigs were positive (Winkelman 1996b). Bronsvoort et al. (2001) found a strong positive association between LI seropositivity of grower-finisher pigs and the seropositivity of their dams. Positive LI sero-status of the sows is associated with lower LI titre of protective

antibodies in their offspring as a result of lack of exposure and/or higher passive immunity to the organism (Winkelman 1996b). The ability to induce a LI infection depends on the young pig's titre of maternal antibodies (Bona and Bilkei 2003). Effective length of protection provided by maternal passive immunity for the piglets has not yet been established (Gebhart and Guedes 2001). According to Gebhart and Guedes (2001), piglets having maternal antibody protection have better outcomes under LI challenge and shed the bacteria for a shorter period compared to piglets born to LI-naïve gilts.

Bronsvoort et al. (2001) identified three factors that influence the LI serological status of the breeding units (LI serological status of an adjacent fattening unit, farrowing house management, and percentage of multiparous sows). The same authors (Bronsvoort et al. 2001) identified four factors that influence LI serological status in a grower-finisher unit (LI serological status of the breeding unit, number of pigs entering the grower-finisher site within the last 6 month, percentage of pigs housed on concrete slats, and outdoor rearing). Because sow management, housing and nutrition were identical in the present trial, the sows' seropositivity to LI but not the above mentioned management factors (Bronsvoort et al. 2001) might have exerted the highest influence on LI seroprevalence in their offspring.

Retrospectively it seems that IFA testing of the offspring of LI seropositive sows should have been performed before challenge. Nevertheless, as maternal antibodies will be excreted both in colostrum and milk of the sow, LI seropositivity of the offspring of LI seropositive sows must be expected.

Séropozitivita prasnic na *Lawsonia intracellularis* (LI) ovlivňuje růst a séropozitivitu potomstva na LI

Cílem studie bylo otestovat, zda séropozitivita k *Lawsonia intracellularis* klesá rychleji u selat séropozitivních matek než u selat matek LI-negativních v chovech infikovaných porcinní proliferativní enteropatií (PPE). V chorvatském velkochovu byly testovány prasničky v pozdní gestaci pomocí nepřímého průkazu sérových protilátek (IFA). Testováno bylo 20 prasnic LI-pozitivních a 20 LI-negativních. Jejich 2 týdny starým selatům (191 od LI-pozitivních matek a 192 od LI-negaitvních matek) byla intragastricky podána čistá kultura *L. intracellularis.* Vzorky krve na stanovení protilátek jim byly odebrány v 5., 10., 15., 20. a 25. týdnu věku.

Séropozitivita u potomstva séronegativních matek byla nejvyšší v 5 týdnech (85,9 %) a postupně klesala od 10. do 25. týdne na pouhých 6,3 %, zatímco u selat séropozitivních prasnic byla séroprevalence nižší a rychleji odeznívala: 21,4 % v 5. týdnu a 0 %v 15., 20. a 25. týdnu. Ve výskytu průjmů nebyly mezi selaty obou skupin signifikantní rozdíly. Mortalita byla signifikantně ($p < 0,05$) vyšší (20,4 %) u selat séronegativních prasnic ve srovnání s potomstvem IFA-pozitivních matek (14,1 %). Průměrné přírůstky hmotnosti byly vyšší ($p < 0,05$; 741 ± 44 g vs 611 ± 41 g.d^{-1}) u selat IFA- pozitivních matek ve srovnání se séronegitvními. Stěžejním poznatkem této práce je, že séropozitivita k LI klesá rychleji u běhounů narozených séropozitivním prasnicím než prasnicím séronegativním v chovech s endemickým výskytem porcinní proliferativní enteropatie (PPE).

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