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*Review Articles*

# Known Facts About the Epidemiology of Porcine Proliferative Enteropathy

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## Abstract

**The amount of new information of the last seven years regarding *Lawsonia intracellularis* sensitivity to antimicrobial drugs, prevention/control based on treatment and vaccination, diagnosis and, finally, genome is amazing. Nevertheless, not much is known about the epidemiology of the disease, mainly regarding the source of infection, resistance of the bacteria in the environment and possible biological vectors that could spread the infection among farms. It was learned that as few as 1,000 to 10,000 bacteria are enough to induce infection and fecal shedding two to three weeks after inoculating susceptible pigs. It was also learned that *L. intracellularis* organisms can survive at least 14 days in infected feces at room temperature. Sow to piglet transmission is also speculated as a possible and reasonable method of transmission, however, detection of the bacteria in sow feces, quantification of these shed bacteria and evaluation of susceptibility of suckling piglets have never been done. There is no information in the literature regarding the importance of fomites such as boots and biological vectors for the transmission of *L. intracellularis*. Risk factors related to proliferative enteropathy have been published using retrospective and case control studies showing that transportation, feed change, significant temperature variation, pig flow and floor are all important aspects that have to be considered in order to reduce the pressure of infection. Nevertheless, even applying this knowledge in association to the use of efficient antimicrobials, there have been not many successful attempts to eradicate *L. intracellularis* from pig herds. It is clear that there is a lot missing about transmission and resistance of the bacteria in order to make a better and sufficient assessment of the relevant aspects of an eradication program.**

**Keywords :** Swine, *Lawsonia intracellularis,* proliferative enteropathy, epidemiology

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## Introduction

Proliferative enteropathy (PE) is an enteric disease that occurs in pigs (McOrist and Gebhart, 2006) and in a number of other species (Cooper, 1996). The etiologic agent of PE in swine is the obligatory intracellular bacterium *Lawsonia intracellularis* (Gebhart et al., 1993; Lawson et al., 1993; McOrist et al., 1993; McOrist et al., 1995). The disease in pigs, commonly referred to as ileitis, has two major clinical aspects, acute hemorrhagic diarrhea and sudden death of replacement animals and finishing pigs close to be marketed, known as proliferative hemorrhagic enteropathy (PHE); and chronic mild diarrhea and reduced performance in grow-finishing pigs, known as porcine intestinal adenomatosis (PIA) (Lawson and Gebhart, 2000; McOrist and Gebhart, 2006).

PE is widespread in swine herds (30-93% are infected) of different production systems and in all parts of the world (Chang et al., 1997; Kim et al., 1998; Chiriboga et al., 1999; Stege et al., 2000, 2004; Biksi et al., 2007). Serologic studies have shown prevalence of PE positive herds ranging from 60 to 90% in different countries (Lawson et al., 2000). The economic impact of PE to the swine industry was estimated to be very high. It was estimated to cost the industry $20/sow annually in Australia (Lawson and McOrist, 1993), and $20 million annually in the United States (Bronsvoort et al., 2001).

The amount of new information and discoveries of the last six or seven years regarding *L. intracellularis* (Lawson and Gebhart, 2000), sensitivity to antimicrobial drugs, prevention/control based on treatment and vaccination, diagnosis and, finally, genome is amazing (McOrist and Gebhart, 2006). New molecules has shown to be effective against *L. intracellularis* infection *in vivo* (Kyriakis et al., 2002[a,b]) and *in vitro*. A new modified-live vaccine, Enterisol Ileitis (Kroll et al., 2004), was developed and launched internationally and a lot was learned about the dynamic of the disease performing seroprofiles of herds (Lee et al., 2001; Chouet et al., 2003; van der Heijden et al., 2004; Stege et al., 2004; Paradis et al., 2007). The complete bacteria genome sequence was accomplished in 2003 at the University of Minnesota (Gebhart et al., 2003) and it opened an enormous area of research and new ways of understanding better this fastidious organism. Nevertheless, we still do not know much about the epidemiology of the disease, mainly regarding the source of infection, resistance of the bacteria in the environment and possible biological vectors that could spread the infection among farms. Our intention in this review is to discuss some aspects related to what is known about epidemiology of proliferative enteropathy.

### Bacteria characterization

Due to the fact that it is not possible to cultivate *L. intracellularis* in conventional bacteriology media, final classification of the organism had to be done by molecular taxonomic methods. Gebhart et al. (1993), using the recently developed molecular taxonomic procedure of 16S rDNA sequence analysis (Weisburg et al., 1991), showed that sequences obtained from organisms purified from the ileal mucosa of four pigs were similar to those of *Desulfovibrio desulfuricans* (91% similarity). In another study, a sequence comparison showed 92% similarity between *Bilophila wadsworthia,* a free-living anaerobic human pathogen, and *L. intracellularis* (Sapico et al., 1994). Finally, in 1995 this intracellular bacterium, previously known as *Campylobacter-like organism* (CLO), *Ileal symbiont intracellularis* and *Ileobacter intracellularis*, was established in a new genus as *Lawsonia intracellularis* (McOrist et al., 1995).

*L. intracellularis* is a Gram-negative curved or sigmoid shaped rod, 1.25 to 1.75 μm long and 0.25 to 0.43 μm wide. The bacterial wall has a trilaminar outer envelope, frequently separated from the cytoplasmic membrane by an electron-lucent zone. No fimbriae or spores have been detected. A long, single, unipolar flagellum has been observed by electron microscopy in three different cell culture-grown isolates (Lawson and Gebhart, 2000).

Despite the fact of causing two different clinical presentations in swine and been detected in several animal species (Table 1), significant differences among *L. intracellularis* isolates have not been demonstrated or correlated with these clinical parameters. An initial study comparing the outer membrane proteins of the six most diverse *L. intracellularis* isolates did not showed any significant difference among them (Guedes and Gebhart, 2003[b]). In this study, the most diverse isolates available were used. Two of these isolates had been extracted from pigs with the acute form of the disease, other two extracted from pigs with the chronic form, one isolate from a foal and the last one from a hamster. Among the four pig isolates, two were from Europe and two from US. Based on these results, it is fair to conclude that there is no phenotypic difference among *L. intracellularis* isolates, it is not possible to differentiate them by serology and there is homologous humoral immune response induced by different isolates.

Evaluation of the 16S-23S intergenic spacer regions of different *L. intracellularis* isolates resulted in no significant variation among the isolates mentioned above (Al-Ghamdi, 2003). Sequences obtained from *L. intracellularis* isolates from pigs and foal were 100% similar. The hamster isolates shared 99.8% similarity with the foal and pig isolates. In the same study, different molecular techniques such as REP-PCR *(Repetitive Element based Polymerase Chain Reaction)*, using REP and ERIC primers, and AFLP *(Amplified fragment length polymorphism)* were applied to the same *L. intracellularis* isolates. Three subtypes of *L. intracellularis* isolates, different among the molecular techniques used, were demonstrated; however, no correlation with clinical presentation was observed.

**Table 1.** First reports of natural occurrence of proliferative enteropathy in different animal species

| Species | Reference |
|---|---|
| Pig | Biester and Schwarte, 1931 |
| Hamster | Jonas et al., 1965 |
| Lamb | Cross et al., 1973 |
| Guinea pig | Elwell et al., 1981 |
| Blue fox | Landsverk, 1981 |
| Horses | Duhamel and Wheeldon, 1982 |
| Ferret | Fox et al., 1982 |
| Rat | Vandenberghe and Marsboom, 1982 |
| Rabbit | Umemura et al., 1982 |
| Dog | Collins et al., 1983 |
| Deer | Hill et al., 1987 |
| Ostrich | Cooper et al., 1997[a,b] |
| Emu | Lemarchand et al., 1997 |
| Rhesus macaque monkey | Klein et al., 1999 |
| Mice | Abshier et al., 2001 |

After the genome sequence of the bacterium has been completed, four sets of primers targeting hypervariable areas of the genome were design and used for the VNTR *(Variable Number of Tandem Repeats)* technique (Beckler and Gehbart, 2005). The evaluation of the number of tandem repeats in the product of each primer reaction, applied in different *L. intracellularis* isolates, provided a specific profile for each isolate. This profile showed to be stable within an isolate in different time points and under different conditions. More and more data has been compiled from different VNTR profiles of different PE cases at the University of Minnesota. This technique has shown to be an interesting tool for molecular epidemiologic studies. Nevertheless, these molecular differences are likely not related to pathogenicity of *L. intracellularis* isolates, as these areas are not coding targets.

**Resistance in the environment**

Information about survival and resistance of *L. intracellularis* in the environment is scarce. A unique investigation into this area (Collins et al., 2000) found that intestinal colonization of pigs by *L intracellularis* was detected after they had been orally inoculated with feces from positive animals. These animals received infected feces that had been stored for 2 weeks at temperatures between 5 and 15ºC. An interesting point that is not usually mentioned in reviews or book chapters is the fact that the other groups of animals in this study were orally inoculated with *L. intracellularis*-infected feces, either on the day the feces were collected, or after storage for 1 or 5 weeks at the same temperatures. As a result, the correct conclusion is that the bacteria can survive temperatures raging from 5 to 15ºC for at least 2 weeks. There is a gray period from 2 to 5 weeks in the environment, when the survival capability was not tested.

In this same article (Collins et al., 2000), *in vitro* studies with pure cultures of *L. intracellularis* demonstrated full susceptibility to a quaternary ammonium disinfectant (3.3% cetrimide), less so to 1% povidone-iodine, but not susceptible to 1% potassium peroxymonosulfate or a 0.33% phenolic mixture.

**Transmission**

Feces from infected pigs are the main source of new infections in susceptible animals (McOrist and Gebhart, 2006). Pig-to-pig contact has been shown to be an important via of transmission. Rowland and Rowntree (1972) showed an association between the PHE outbreak in the young breeding stock and the onset of chronic diarrhea in contact weaned pigs a few weeks after. *L. intracellularis* infection was transmitted between breeding stock animals to young adult pigs in a natural PHE outbreak, where movement of sows and boars between units was permitted (Love et al., 1977). In an experimental trial, sentinel pigs became infected when housed in contact with pigs experimentally inoculated with pure culture of *L. intracellularis* (Jordan et al., 1997). It is important to remember the bacteria can survive in the environment for at least 2 weeks (Collins et al., 2000), as mentioned above.

Sow to piglet transmission is always speculated as a reasonable method of transmission. A serologic study performed in 184 herds showed that seropositivity in the breeding herd is an important risk factor for new PIA cases in growing-finishing pigs. In addition, seropositivity in growing-finishing animals was also a risk factor for the acute form of disease (PHE) in replacement animals (Bronsvoort et al., 2001). A study of the prevalence of *L. intracellularis* in fecal samples detected by PCR in an endemically infected herd in Europe showed the highest proportion of positives among pigs between 10 and 24 days after weaning (Moller et al., 1998). Only 12.9% of the grower and finishing animals and 0.9% of the mature animals were positive. In the same study, the possible epidemiological importance of infection in breeding sows and young sucking piglets was indicated. Fecal shedding have been reported as early as in three-week-old piglets (Lopez et al., 2000) and piglets between 25 and 42 days of age (Moreno et al., 2000). All the above data give some

indication of sow to piglet transmission as a possible mechanism of dissemination of infection. However, detection of the bacteria in sow feces, quantification of these bacteria shed and evaluation of susceptibility of suckling piglets have never been done. As a result, protocols of antibiotic use for sows at the end of gestation and during lactation in order to reduce bacterial shedding have no scientific support yet.

Fomites such as boots and biological vectors such as birds and mice are often present in marketing folders about *L. intracellularis* cycle of infection. Despite the real possibility of these events, so far, the nearest that we have gotten to these conclusions are the survival of the bacterial in feces for up to 2 weeks (Collins et al., 2000), the report of natural infection of a colony of mice at the University of Missouri, in US (Abshier et al., 2001), and reports of natural disease in ostrich (Cooper et al., 1997[ab]; Cooper & Gebhart, 1998) and emu (Lemarchand et al., 1997). Two attempts to detect natural infection (McOrist et al., 2003) and susceptibility to experimental infection (Collins et al., 1999) of chickens (Gallus domesticus) resulted in negative results. There was no attempt of detection of *L. intracellularis* DNA in dirt boots from positive farms; there was no attempt of testing the possibility of transmission of *L. intracellularis* from experimentally inoculated mice to susceptible pigs; there was no attempt to check presence of *L. intracellularis* in small free birds. These do not mean that these ways of transmission do not happen. It just states that they have not been proved, yet. In fact, we have been trying, in collaboration with the University of Minnesota, to infect free living sparrows, captured in a metropolitan area of Brazil, with pure culture of *L. intracellularis* in order to evaluate if this ubiquitous Passeriform bird is susceptible to the infection and can shed this organism in the feces. Preliminary results of this study have resulted negative.

**Infecting dose and duration of shedding**

The minimal infecting dose of *L. intracellularis* sufficient to infect and induce shedding in exposed animals has never been definitively estimated. The closest that we got was reported by Dr. Collins from Australia (Collins et al., 2001). As demonstrated in table 2, they had five different groups of pigs that were challenged with amounts of bacteria varying form $10^3$ to $10^{10}$, and a non-challenged control group. Even in the lower challenged dose ($10^3$ *L. intracellularis* organisms per pig) was enough to induce fecal shedding of the bacteria in challenged animals. However, this fecal shedding in animals inoculated with low doses was delayed up to 56 days of after inoculation.

The duration of fecal shedding is variable and depends on many factors such as load of infection, course of the disease, antibiotic used, and many others. It has been demonstrated, both in experimentally (Guedes and Gebhart,

**Table 2.** Pattern of infection of pigs inoculated with varying dose of L. intracellularis (Collins et al., 2001)

| Groups | Estimated dose of *L. intracellularis* | Days pi when 80% pigs PCR positive | Days pi when 80% pigs IFAT positive |
|---|---|---|---|
| 1 | Not inoculated | 0 | 0 |
| 2 | $2.0 \times 10^3$ | 26-54 days | 56-70 days onwards |
| 3 | $2.0 \times 10^5$ | 19-33 days | 56-70 days* |
| 4 | $2.0 \times 10^7$ | 14-28 days | 35-49 days |
| 5 | $2.0 \times 10^{10}$ | 7-44 days | 21-70 days onwards |

*Only 2 of 5 pigs developed a detectable serological response

2003a) and natural outbreaks (Guedes et al., 2002), that some pigs, called "super-shedders", may eliminate bacteria in the feces up to 12 weeks. It has also been shown that experimentally infected pigs can excrete between $5x10^4$ and $7x10^8$ *L. intracellularis* per gram of feces (Smith and McOrist, 1997). The association among a very low infective dose, very high amount of bacteria shed in the feces, prolonged fecal shedding, and the bacterium survival in the environment for at least 2 weeks demonstrate how difficult it is to control transmission and the spread of infection. Thus it is not surprising how prevalent and disseminated this bacterium and disease are around the world.

**Infection relapse and risk factors for new cases**

A very common question about PE epidemiology regards the occurrence of infection relapse. In other words, if a batch of pigs that has been exposed to the bacteria and has had signs of the disease may be re-infected and has another outbreak. Based on the knowledge available, the answer is no. There is no information or data demonstration relapse of the disease in a group of pigs previously exposed to the bacteria. However, different batches of animals in the same herd may have clinical disease in different time points. Two studies demonstrate and support this statement. Collins and Love (2007) re-inoculated previously infected pigs after *L. intracellularis* fecal shedding had ceased. These animals were observed for clinical signs, fecal shedding and humoral immune response. Neither clinical disease nor fecal shedding were detected. In a second study, one hundred pigs from five herds were followed by fecal PCR every other week from weaning to slaughter. Peak of shedding occurred between 10 and 12 weeks of age, and lasted in average 2 to 6 weeks. After 18 weeks of age all shedding had ceased and re-infection at PCR detectable level was not seen (Stege et al., 2004).

Risk factors related to new cases of proliferative enteropathy have been published using retrospective and case control studies showing that transportation, feed change, significant temperature variation, pig flow and slat floor are all important aspects that have to be considered in order to reduce the pressure of infection (Bane et al., 2001; Bronsvoort et al., 2001; Stege et al., 2004). Results from a questionnaire survey among British farm owners indicated that slatted and meshed flooring were important risk factors for PE. They suggested that such floors, commonly found in post-weaning facilities, are often insufficiently cleaned (Smith et al., 1998). However, the findings in this report were based on the owner's opinions and no diagnostic information was used to support the data. Another study, which included a questionnaire survey, production records and fecal PCR analysis, demonstrated that the use of new buildings and recent mixing of pigs were associated with PE (Bane et al., 2001). These findings support a hypothesis that subclinically infected pigs shed *L. intracellularis* in the feces, particularly after stress.

Pig flow will have a direct effect over the age when PE mostly affects the animals. Multisite production systems usually have a delayed infection (12-20 weeks), which may be manifested by chronic or acute PE cases. Farrow-to-finished, continuous flow systems usually have an early exposure (5-7 weeks) that is usually manifested by chronic and subclinic PE problems (Chouet et al., 2003).

The importance of knowing the infection status of replacement gilts regarding *L. intracellularis* was shown in a recent paper from Canada (Friendship et al., 2005). Positive herds that received replaced animals from a Lawsonia-free breed stock herd faced recurrent problems of the acute form of the disease in these incoming animals, while negative commercial herds that received these same background animals never had any problems. Just after an outbreak of the acute form of the disease in the breed stock herd, the scenario completely changed in the commercial herds. The positive ones stooped having problems and the negatives start to have severe outbreaks.

**Final considerations**

There have been many advances in the knowledge about PE epidemiology, mainly due to the improvement of diagnostic tests. Nevertheless, even applying this knowledge in association to the use of efficient antimicrobials, there have been not many successful attempts to eradicate *L. intracellularis* from pig herds. The Danish eradication program has had good results when bringing animals to new facilities, but usually animals are re-infected two year later at the most. It is clear that there is a lot missing about transmission and resistance of the bacteria in order to make a better and sufficient assessment of the relevant aspects of an eradication program. As a result, more studies have to be conducted in order to understand important details about *L. intracellularis* epidemiology.

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