

Review

Advances in Ileitis Control, Diagnosis, Epidemiology and the Economic Impacts of Disease in Commercial Pig Herds

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Abstract: Proliferative enteropathy, commonly known as “ileitis” continues to be a significant production-limiting disease in pig herds throughout the world. The disease can be controlled with a combination of vaccination and antibiotic medication. However, pressure from consumers to reduce antibiotic use in livestock industries highlights the need to better understand the epidemiology of ileitis, the mechanisms of immunity, and to identify management factors that can reduce the load of *Lawsonia intracellularis* in both pigs and the environment. New diagnostic assays and economic modelling of ileitis will help producers target optimal treatment times and minimize the production losses associated with ileitis. This review aims to outline the current advances in disease diagnosis, epidemiology, control strategies and the economic impact of both clinical and sub-clinical disease.

Keywords: animal diseases; epidemiology; economic impact; ileitis; proliferative enteropathy; *Lawsonia intracellularis*; transmission; immunity; diagnosis

1. Introduction

Ileitis is the common name for the wasting disease in pigs more accurately named porcine proliferative enteropathy (PPE). Clinical signs include reduced weight gains and diarrhea in growing pigs. Finisher or breeding animals can be affected with the more acute proliferative hemorrhagic enteropathy (PHE), losing blood in their feces due to intestinal hemorrhage. The proliferative enteropathies encompass a group of conditions (necrotic enteritis, regional ileitis, PHE and porcine intestinal adenomatosis) with similar pathogenesis. Gross pathology presents as a thickened mucosa in the distal small intestine and proximal large intestine. Histopathology is characterized by proliferation

of crypt enterocytes, loss of goblet cells and intracellular bacteria in the apical cytoplasm of proliferating enterocytes [1]. The obligate intracellular bacterium, *Lawsonia intracellularis*, is the etiologic agent of all forms of PPE [2].

The characteristic gross and histological lesions of proliferative enteropathy (PE) were first described in North American weaned pigs in 1931 [3]. Subsequent reports described lesions of PPE in Europe, Asia, Australasia and America over the next 40 years [4–10]. However it was not until 1973 that the presence of intracellular bacteria within proliferative lesions was first described [11]. It took another 20 years for the etiologic agent, *L. intracellularis*, to be cultured in mammalian cells and for Koch's postulates to be fulfilled with the reproduction of PE in pigs [2,12].

Pigs of all ages, and pigs housed in a range of management systems, including farrow to finish farms and multiple site production farms are affected with PE. However, it is predominantly in grower and finisher pigs where disease control strategies are focused. Antibiotics were commonly used to treat or prevent PE, and practices developed where pigs were medicated continuously from about nine weeks of age until slaughter. While no data has been published to suggest that *L. intracellularis* develops resistance to commonly used antibiotics, the overuse of antibiotics could lead to antibiotic resistance in other bacteria. The aim of this review is to detail the advances in PE epidemiology, diagnosis and control strategies that will help reduce the reliance on antibiotics to control PE.

2. The Prevalence of Proliferative Enteropathy

The estimated prevalence of disease and *L. intracellularis* infection depends on the diagnostic method used. Slaughter checks relying on gross pathology have suggested that somewhere between 5% and 20% of pigs are affected with PE [13–17]. However, slaughter checks significantly underestimate the incidence of PE in grower pigs because PE lesions resolve within two to four weeks of clinical signs being present [18]. Producers and veterinarians surveyed in the UK and Australia estimated that clinical disease was prevalent in somewhere between 30% and 56% of herds [19,20]. Serological surveys of finisher pigs estimated that more than 80%, and up to 100% of pigs are infected with *L. intracellularis* [21–23]. In contrast, detection of *L. intracellularis* DNA in feces (by polymerase chain reaction) suggests that between 4.5% and 45% of herds are infected with *L. intracellularis* and the prevalence of infected animals ranges between 3.3% and 12% [24–29]. The diagnostic tests also vary in their ability to diagnose clinical disease, sub-clinical disease or *L. intracellularis* infection. The ability to interpret the results of infection prevalence studies in herds will help producers target disease control strategies to the right age group of pigs, and reduce the unnecessary use of antibiotics. Strategies to ensure the rational use of antibiotics are outlined in section 4.1.

3. Diagnosis of Proliferative Enteropathy

Early diagnosis of PE relied on gross pathology at the abattoir (thickening of the ileal mucosa) and histopathology; visualizing the proliferative epithelium and intracellular curved bacteria in the apical cytoplasm of cells with a Warthin Starry silver stain, and later with a specific monoclonal antibody to *L. intracellularis* [30,31]. Necropsies to diagnose PE on farm were only undertaken on sick pigs, not expected to recover, so other diagnostics were needed. The development of ante-mortem diagnostic assays for *L. intracellularis* infection was aided by the culture of *L. intracellularis*.

3.1. Serological Assays

Relatively impure *L. intracellularis* proteins, extracted from PE affected tissue, were initially used in both an ELISA and an indirect fluorescent antibody test (IFAT) to detect serum IgG antibodies to *L. intracellularis* [32,33]. The IFAT was able to overcome high background problems observed in the early ELISA by visualizing specific antigen-antibody reactions under the microscope, allowing differentiation between fluorescing *L. intracellularis* and non-specific reactions. Culture of *L. intracellularis* allowed the development of whole cell immunoperoxidase antibody tests (IPMA) [34] and ELISA tests using *L. intracellularis* outer membrane proteins or lipopolysaccharides (LPS) as antigens [35,36], but most of these assays have remained as research tools. A blocking ELISA for serum IgG to *L. intracellularis* (bioScreen Ileitis antibody ELISA; Munster, Germany) is now commercially available and routinely used to determine the timing and prevalence of *L. intracellularis* infection. Serology can help producers and veterinarians identify risk factors and the efficacy and optimal timing for PE control measures including vaccination, medication and improved hygiene [37–39]. The sensitivity of this blocking ELISA was between 72% and 90.5% and the specificity was between 83% and 93%, using sera from both field and experimental challenge trials [40,41]. Regardless of which serological assay is used, *L. intracellularis* antibodies are not routinely detected until about 21 days post challenge, after clinical signs and fecal shedding are already observed, so serology is an indicator of previous infection [42,43]. The IFAT, IPMA and LPS ELISA have been modified to measure *L. intracellularis* specific IgA and IgM antibodies in sera and in mucosal scrapings, so they continue to be used to elucidate the immune response to both vaccination and virulent challenge [44–46].

3.2. Molecular Assays

The development of DNA based diagnostic assays such as hybridization and the polymerase chain reaction (PCR) did not rely upon the culture of *L. intracellularis*. Hybridization probes were produced from short fragments of *L. intracellularis* DNA that were cloned [47] and used to detect *L. intracellularis* DNA in pig faeces. Later, short fragments of *L. intracellularis* DNA were amplified by PCR using oligonucleotides synthesized to detect *L. intracellularis* DNA [48–51]. The relatively short duration of *L. intracellularis* excretion (up to three weeks) and the higher cost of PCR (compared to serology) has meant that the PCR has been used primarily to detect infection in diarrheic pigs, and is less useful for monitoring PE control in herds. The PCR assay cannot discriminate between live and dead *L. intracellularis* in feces, so some reports of PCR positive pigs detected at a single time point make it difficult to interpret whether the pigs were actively infected at the time or if *L. intracellularis* was transiting through their digestive tracts.

Although fecal PCR is both more sensitive and specific than other diagnostic assays, it was not quantitative until recently, partly because the amplification of *L. intracellularis* DNA can be inhibited by the presence of large amounts of competing non-target DNA [52], bile salts and bilirubin in feces [53]. The interpretation of fecal PCR results has also been confused by the sensitivity of the assay. Detection of *L. intracellularis* DNA does not prove that *L. intracellularis* is the cause of

diarrhea. The development of a multiplex PCR assay that can detect *L. intracellularis* and *Brachyspira* spp. in the same sample helped identify multiple causes of diarrhea in the same animal [54,55].

Recently a number of quantitative real time PCR (qPCR) assays have been developed for *L. intracellularis* [56–59]. Sensitivity of these new assays was aided by advances in DNA extraction protocols with magnetic particle processors capable of capturing nucleic acids and removing them from inhibitors in the feces. These qPCR assays can calculate *L. intracellularis* numbers in feces relative to standards seeded with known numbers of *L. intracellularis*, providing a reproducible linear range of 10^8 to 10^4 bacteria/g feces. The number of *L. intracellularis* excreted in pig feces (determined by qPCR) is between 10^4 and 10^8 *L. intracellularis* per gram of feces depending on whether pigs are clinically or sub-clinically affected with PE [56–59], with clinically affected pigs excreting more than 10^7 *L. intracellularis* per gram of faeces.

L. intracellularis excretion levels correlated well with antibody titres, the severity of histopathology lesions, the duration of *L. intracellularis* excretion and the severity of diarrhea [60,61]. A weaker inverse correlation exists between the qPCR and production losses associated with PE, which may be explained by other factors that impact on pig growth. However, a one \log_{10} unit increase in the number of *L. intracellularis* increases the risk for low growth rate by two times [61]. The critical number or threshold of *L. intracellularis* that causes reduced growth rates in experimentally infected pigs is reported to be above 10^7 *L. intracellularis* per gram of feces [62]. Once the number of *L. intracellularis* shed by pigs increased from 10^7 to 10^8 per gram of feces, average daily gain was reduced by 131 g/day. Identifying a critical threshold of infection across a wide range of commercial management systems will allow producers to use the qPCR to target ileitis treatment options.

Preliminary studies have indicated that fecal samples can be pooled prior to qPCR analysis to help reduce costs and to encourage producers to use the qPCR as a herd health monitoring test. Theoretical pooling of samples indicated that the qPCR was still accurate when pools of 10 individual samples were used [63]. However, pools of five fecal samples provided more accurate results than pools of 10 feces when the qPCR assays were performed [64].

The diagnosis of *L. intracellularis* infection in real time by qPCR enables producers to quantify the efficacy of PE control measures, as reduced numbers of *L. intracellularis* correlate with reduced severity of PE. Data from experimental challenge trials shows the significant reduction in the number of *L. intracellularis* shed in pigs vaccinated with Enterisol[®] Ileitis or medicated with antibiotics [46,65]. The qPCR can also be used to quantify the efficacy of disinfection and hygiene protocols [39] and the biosecurity risk associated with rodents near piggeries [58]. Avoiding the over-use of antibiotics for the control of PE relies on the correct interpretation of all of the new diagnostic assays. Quantitative assays identify the severity of disease and help producers decide on what level of intervention is needed to control PE. Qualitative assays such as serology provide producers with information on the optimal timing of disease control measures and the prevalence of exposure to *L. intracellularis* in the herd.

4. Control of Ileitis

The development of experimental PE challenge models and diagnostics to monitor *L. intracellularis* infection enabled antibiotic efficacy studies to be performed in the absence of concurrent infections

commonly found on farm. Prior to this, veterinarians relied on monitoring clinical signs of PE or pathology at slaughter to determine whether treatment strategies were effective. However, diarrhea and poor growth could be caused by other factors and suspect gross pathology at abattoir was only proven by histopathology in less than 11% of cases [14]. These new disease challenge models enabled the more rational use of antibiotics for PE control, and allowed evaluation of other potential control strategies including immunization and improved hygiene.

4.1. Antibiotic Medication

The efficacy of antibiotics such as olaquinox, to control PE was originally identified by field veterinarians trying to control outbreaks of PE [66]. Later experimental challenge trials demonstrated that high doses of tiamulin, tylosin, chlortetracycline, lincomycin and olaquinox were able to treat pigs with PE, reducing clinical signs, histological lesions of PE and the duration of fecal shedding of *L. intracellularis* [67–74]. High doses of tiamulin, tylosin, olaquinox and chlortetracycline could also prevent *L. intracellularis* infection if given continuously in feed [67,70,74]. However, once these antibiotics were removed, the pigs remained immunologically naïve and were susceptible to a later *L. intracellularis* challenge [75]. In addition, continuous use of antibiotics may increase the potential for other bacteria to develop resistance.

Public concern over the development of antibiotic resistance by bacteria led to significant pressure world-wide to find alternative strategies to control PE and to reduce the prophylactic use of antibiotics. Protective immunity to PE was first demonstrated in a minimal disease piggery with two sequential outbreaks of the hemorrhagic form of ileitis (PHE). Pigs that survived the first outbreak were protected from a subsequent disease outbreak [76]. The authors proposed that immunity could be induced by bringing young animals into contact with the source of infection for three weeks, prior to the addition of antibiotics to terminate infection.

Protective immunity was later demonstrated in experimental challenge models, where pigs previously challenged orally with *L. intracellularis* were protected from re-infection on a subsequent challenge [43,45,77]. The failure to detect *L. intracellularis* in the feces of re-challenged pigs by PCR indicated that virulent, live *L. intracellularis* in the second inoculum were not able to colonize mucosal cells in previously challenged pigs. Therapeutic strategies were designed to allow *L. intracellularis* infection and immunity to re-infection, with antibiotics used to avoid clinical disease. Antibiotics were removed from pig diets for 12 to 18 days and then pulsed in feed or in water at high doses for two to four days every two to three weeks to suppress disease. The period without antibiotics allowed pigs to become infected with *L. intracellularis* but also to develop immunity to re-infection [78].

Antibiotics, along with extensive cleaning of pens and depopulation of younger pigs were also used in attempts to eradicate PE, by eliminating *L. intracellularis* from the pig and the environment [79–82]. Eradication of clinical signs of PE appeared possible on smaller farms, but very few studies were able to demonstrate complete eradication of *L. intracellularis* from the herd and the environment over time. Biosecurity needed to be maintained at a very high level to ensure continued eradication of *L. intracellularis* [82]. Outbreaks of severe disease often followed when *L. intracellularis* was accidentally introduced into a naïve herd [80].

4.2. Improved Hygiene

L. intracellularis can survive (outside of the pig) in conventional pens for at least two weeks at temperatures between 9 °C and 18 °C [39,50]. Management practices that improve biosecurity and hygiene, including all-in-all-out production and minimal mixing of pigs, are associated with a lower risk of *L. intracellularis* infection [83–85]. The housing and flooring in pens may also impact on the survival of *L. intracellularis*. It may be expected that pigs reared on deep litter systems would be exposed to greater numbers of pathogens, including *L. intracellularis*, because deep litter systems are only cleaned and/or disinfected between batches of pigs, compared with conventional pens where manure is removed more regularly. However, pigs grown out on deep litter systems had a similar prevalence of *L. intracellularis* infection as cohorts raised on traditional concrete-based systems [86], and grower and finisher pigs reared on partially slatted floors had a higher risk of diarrhea than pigs housed on solid concrete floors [87]. Power hosing concrete slatted floors with cold water, followed by disinfection with potassium peroxymonosulfate (Virkon S) and drying time was able to significantly reduce the survival and transmission of *L. intracellularis* from the environment to naïve pigs [39]. The efficacies of a wide range of other disinfectants against *L. intracellularis* have been tested by *in vitro* culture methods. The iodophore povidone iodine and quaternary ammonium compounds were both effective against *L. intracellularis* in *in vitro* studies, in the absence of faecal material [50,88]. However, the efficacy of disinfectants needs to be studied in pig pens with residual manure, which can inactivate the disinfectant. Reducing the number of *L. intracellularis* in pens between batches of pigs reduces the severity of disease in subsequent batches, but the aim is not to remove all *L. intracellularis*, or naïve herds may develop, which are highly susceptible to outbreaks of PHE, the more acute form of PE.

4.3. Vaccination and Induced Immunity

Oral vaccination with a commercial avirulent live *L. intracellularis* vaccine (Enterisol[®] Ileitis) protects pigs from clinical disease; significantly reducing microscopic lesions of PE following a virulent heterologous *L. intracellularis* challenge [89]. Vaccination did not completely prevent fecal shedding of *L. intracellularis* following virulent challenge, but did significantly reduce the number of *L. intracellularis* shed in feces and the duration of shedding in vaccinated pigs [42,89,90]. In many commercial herds antibiotic medication was no longer required to control PE in vaccinated herds. However, producers believed that in some straw-bedded systems *L. intracellularis* infection was harder to suppress with vaccination alone [91]. In these cases, antibiotics were used in tandem with vaccination.

As expected for an intracellular bacterium, *L. intracellularis* infection induces both specific humoral (IgA, IgG) and cell mediated (IFN- γ) immune responses in serum and the intestinal mucosa. The immune response to both natural challenge and oral vaccination is dose-dependent [43–46]. The absence of an anamnestic serum IgA response in re-challenged pigs, along with an increased gamma IFN response, led to the speculation that cell-mediated immune responses alone were likely to be mediators of protective immunity to *L. intracellularis* [45]. However, accumulation of IgA in proliferating crypt cells and in the cell debris of the crypt lumen of PE-affected pigs, where *L. intracellularis* also reside [92], suggests that IgA is also involved in a protective immune response

to *L. intracellularis*. Induction of mucosal immunity is believed to depend upon presentation of the antigen at the mucosal surface, but protection against other intracellular pathogens (*Salmonella enteritidis*) following systemic vaccination [93] suggest that systemic vaccination may protect pigs from *L. intracellularis* challenge. In early studies, disease severity was significantly reduced in pigs vaccinated twice intramuscularly (IM) with *L. intracellularis* bacterin or recombinant GroEL-like protein [94]. More recently, IM vaccinations, with *L. intracellularis* bacterin or recombinant outer membrane proteins of *L. intracellularis* (19/21 and 37 kDa antigens), protected pigs from PE [95]. In comparative studies with the commercial live *L. intracellularis* vaccine (Enterisol[®] Ileitis), vaccination (10-times dose) administered orally or intra-muscularly induced a similar protective immune response, with systemic and mucosal *L. intracellularis*-specific antibodies and mucosal cytokines [46]. These inactivated *L. intracellularis* vaccines appear to protect against infection if sufficient *L. intracellularis* antibodies are present to prevent infection at the mucosal. Alternate adjuvants or higher doses of *L. intracellularis* antigens may increase the efficacy of systemic vaccination routes. To date no detectable immune marker for protection has been identified, so veterinarians have to rely on detection of exposure to *L. intracellularis* (serum IgG antibodies) as a proxy for immunity.

The need to remove all medication for three days before and after vaccination was initially seen as an obstacle to the uptake of an oral vaccine [91]. The vaccine was registered for weaner pigs that may have concurrent infections requiring antibiotic medication. While vaccination pre-weaning may overcome this obstacle, the potential for maternal antibodies to interfere with vaccination was a concern [91]. While maternally acquired antibodies are vital in protecting piglets from pathogen invasion, they may also interfere with oral vaccination. Maternal antibodies ingested by piglets bind to antigens found in the intestinal lumen, form complexes and are then internalized by antigen-presenting cells. Both B and T cells are stimulated in the piglet, though B cell stimulation is inhibited. Modifying the vaccination route may overcome the potential interference of maternal antibodies. Earlier studies demonstrated that IM vaccination could induce a protective immune response in *L. intracellularis* challenged pigs [46,94,95]. However, the Enterisol[®] Ileitis vaccine is not registered for this use.

5. Production and Economic Impacts of Ileitis

The clinical impact of *L. intracellularis* infection depends on the dose of bacteria ingested by pigs [43,96], but also on the expression of bacterial virulence genes [97], stimulation of the host's immune system [98] and environmental factors. The pig's diet, genetics, immune response, intestinal microflora and intestinal health are all likely to impact on the severity of disease.

Clinical signs of ileitis including diarrhea are evident for one to three weeks, reducing feed conversion efficiency (FCE) by up to 50% [99]. Most pigs recover from clinical disease within four to six weeks but chronic disease can affect pig growth over 10 weeks. Significant variation in weight gains has also been observed in pigs affected by PE, with reductions in average daily gain (ADG) ranging from 17% to 84% of the gain of unaffected pigs [99]. This variation in growth within a batch of pigs can lead to increased days to slaughter or increased back fat on larger animals and ultimately economic losses for the producer [100]. Simulation of two different clinical outbreaks of

PE, (reduced feed intake, ADG, feed conversion efficiency, increased P2 back fat) indicated that clinical ileitis costs between \$7 and \$13 AUD for every pig [100,101].

Many pigs are sub-clinically affected, as demonstrated by the extremely high prevalence of seropositive pigs relative to the small proportion of pigs with PE lesions [17,21,23]. Diarrhea is rarely observed in pigs sub-clinically affected with PE, but reductions in ADG between 9% and 42%, and reduced FCE between 6% and 37% have been reported [99,102]. The economic impact of sub-clinical PE is difficult to estimate because many producers are unaware that sub-clinical PE is present in their herd. Experimental reproduction of sub-clinical ileitis in a group of grower pigs caused significantly decreased feed intake (4 kg) over a three week period, but did not reduce pig weights or weight gains in the same period, though variation in pig weights was significantly increased in infected pigs relative to non-infected pigs [103]. In the three weeks after infection peaked, *L. intracellularis* infected pigs increased weight more quickly than in the early infection period, but muscle deposition was significantly reduced [104]. This may in part explain the increased P2 back fat depth reported in previous studies, although no significant differences in body composition, measured with a CT scanner, were observed between sub-clinically and uninfected animals [104]. Economic modeling of these production losses on a herd basis in the absence of control strategies resulted in an \$8.33 AUD reduction in revenue per pig in a herd with 80% of pigs sub-clinically affected [100].

6. Epidemiology of Ileitis

6.1. Transmission of Infection between Pigs and the Environment

The occurrence of PE in segregated early weaning systems indicated that pigs are either infected from the sow pre-weaning, from the environment, or from contact with pigs or other species of animals, birds or insects. Transmission of infection between in-contact pigs was demonstrated in natural outbreaks of PE when animals were recently mixed, or when new animals were introduced [6,10] and also in experimental challenge trials [50,105]. The transmission of *L. intracellularis* to naïve pigs is primarily via the ingestion of contaminated feces. Pigs affected with PE shed large numbers of *L. intracellularis*, enough to infect naïve pigs in contact with them [43,50,105,106]. Epidemiology studies have identified frequent mixing of pigs, the introduction of new pigs and continuous flow production systems as risk factors for PE [22,87].

The detection of *L. intracellularis* in pig feces coincides with the presence of clinical signs of PE [43,107]. However, in some experimental challenge trials, pigs continued to shed *L. intracellularis* after clinical signs of PE ceased, [42,108], suggesting a carrier state similar to Salmonella infections, where stressed carrier pigs re-commence excretion of Salmonella [109]. *L. intracellularis* bacteria were found in the tonsils of pigs clinically affected with PE [110], however, a carrier state of *L. intracellularis* infection has not been demonstrated. Pigs allowed to recover naturally from PE do not continue to shed detectable numbers of *L. intracellularis*, and are not able to transmit infection to naïve, in-contact pigs [111]. Treatment of recovered pigs with the corticosteroid dexamethasone likewise did not lead to detectable *L. intracellularis* excretion, or transmission of infection to naïve in-contact pigs. The development of immunity to *L. intracellularis* infection, even when pigs were

challenged with a much higher secondary dose (increased by four logs), also indicates that *L. intracellularis* does not readily reappear from a carrier state [43,90].

Elimination of *L. intracellularis* from pigs and the environment is important in reducing the transmission of infection to naïve pigs. Chlortetracycline in feed (400 ppm) was able to rapidly terminate *L. intracellularis* excretion relative to tiamulin (200 ppm), oxytetracycline (400 ppm) and non-medicated pigs [65].

6.2. Transmission of *L. intracellularis* from Sows to Their Progeny

The detection of *L. intracellularis* in the feces of a small number of suckling pigs in endemically-infected herds has led researchers to speculate that sows may be a source of infection to their piglets [108,112,113]. However, the detection of antibodies to *L. intracellularis* in three to six week old pigs, and the occurrence of PE predominantly in pigs greater than six weeks of age [32], suggests that piglets are more likely protected from *L. intracellularis* by either antibodies or lymphocytes from the sow in colostrum or later by IgA in milk. In late lactation, circulating antibodies in the sow are concentrated into pre-colostrum, along with circulating IgA-secreting lymphocytes (plasma cells) which “home” to the mammary gland [114]. Piglets can passively absorb maternal antibodies during the first 24 to 36 h of life [115]. Lymphocytes from the birth sow can be absorbed by her piglets in the first week of life [116].

The ability of sows to protect piglets against *L. intracellularis* infection will therefore depend on the level of circulating antibodies in the sow and the transfer of protective immune secretions and cells to her progeny. Three week old piglets suckling on a recently infected sow were immune to challenge with *L. intracellularis*, in contrast to their weaned litter mates that were infected with the same *L. intracellularis* challenge [117]. Four weeks after the protected piglets were weaned they were susceptible to a virulent challenge; whereas their previously infected litter mates were now immune. This passive protection did not persist after pigs were weaned. Sows with no detectable *L. intracellularis* antibodies at farrowing, but from an endemically infected herd, were only able to partially protect their suckling progeny. Clinical signs were reduced, but piglets excreted *L. intracellularis* and raised significantly reduced serum IgG titres to *L. intracellularis* [117]. The ability of sows with higher *L. intracellularis* antibody titres to protect their suckling piglets was also demonstrated in field studies. The progeny of 20 seropositive and 20 seronegative gilts were challenged with *L. intracellularis* at two weeks of age and remained suckling for an additional three weeks. Only 21% of piglets on seropositive gilts had *L. intracellularis* antibodies three weeks post challenge, relative to 86% of piglets from seronegative gilts [112].

6.3. External Vectors in Transmission of *L. intracellularis*

The main source of *L. intracellularis* in the piggery is infected pigs, and transmission is via ingestion of contaminated feces [50,105]. However, outbreaks of PE occur in naïve herds, suggesting that other sources of *L. intracellularis* may exist. A wide range of domestic and wildlife species can become infected with *L. intracellularis* and some also develop PE [118–126]. Birds, insects, rodents and feral pigs are considered the most likely external vectors for PE in pigs due to their proximity to commercial pig units. *L. intracellularis* infection of rodents has been investigated in both field and

experimental challenge studies. Laboratory rats and mice challenged with porcine strains of *L. intracellularis* have been infected, raised persistent immune responses and developed caecal and colonic lesions of PE [58,127–129]. In addition, *L. intracellularis* DNA was detected in the intestinal tissue of wild mice and rats trapped on pig farms where PE was endemic [58,130]. A high proportion of wild rats trapped on pig farms with endemic PE were excreting *L. intracellularis* ($\geq 70.6\%$), but more significantly, a small proportion of these rats excreted extremely high numbers of *L. intracellularis* (10^{10} /g of feces) [58]. Rodents may therefore be an important reservoir of *L. intracellularis* on pig farms, as less than 1 g of rodent feces would need to be ingested by a naïve pig for transmission of infection to occur.

To date the only report of *L. intracellularis* infection in birds relates to ratites including the ostrich and emu [123,124]. As these species are not commonly associated with pig herds, they are unlikely to pose a significant infection risk. Experimental challenge of sparrows with porcine isolates of *L. intracellularis* failed to demonstrate colonization or disease associated with *L. intracellularis* [131]. Conversely, invertebrates collected from a geographical spread of UK pig herds indicated that *L. intracellularis* DNA was frequently detected in house and hover flies, but not in cockroaches [126]. Small differences in DNA repeats (four microsatellite markers) indicated that the *L. intracellularis* isolate found in pigs couldn't be differentiated from the fly isolate. However, it's not possible to say whether the flies are just a measure of *L. intracellularis* infection in the herd or a potential source of infection to naïve pigs.

7. Conclusions

Veterinarians and pig producers have benefitted from the recent knowledge gained in the diagnosis of *L. intracellularis* infection, transmission and survival of *L. intracellularis* and PE control strategies. This also translates to benefits for society, as these recent advances will enable producers to control PE with a reduced reliance on antibiotics.

The development of quantitative diagnostic assays enable producers to measure the severity of *L. intracellularis* infection in pig herds in real time, providing them with opportunities to fine tune treatments and ultimately reduce costs associated with disease control. In the absence of an immune correlate for protection, producers should continue to regularly monitor their herds by serology to ensure that *L. intracellularis* infection is widespread, as a proxy for immunity.

Knowledge of the potential sources of *L. intracellularis* in pigs, external vectors and the environment enables producers and veterinarians to develop hygiene and biosecurity procedures, including rodent eradication programs, to prevent outbreaks of PE. Management strategies that reduce the spread and number of *L. intracellularis* in the pig herd include all-in-all-out production, quarantine areas for newly introduced pigs, minimal mixing of pigs and effective disinfection of pens or sheds between batches.

Vaccination, either with the current live oral vaccine or with a new recombinant protein vaccine given IM, will ensure that all pigs are exposed to *L. intracellularis*, and will be protected from clinical signs of PE on later challenge. Intramuscular vaccination may overcome suggested maternal antibody interference and the need to have a seven day antibiotic-free period in newly weaned pigs. However, in

some instances vaccination alone does not appear to prevent PE. In these cases, careful monitoring of the herd will help target antibiotic medication treatments.

Finally a better understanding of the costs and production losses associated with both clinical and sub-clinical PE will help producers target treatments to appropriate periods in pig production. In conclusion, it is a combination of management practices that will enable control of PE with significantly reduced reliance on antibiotic medication.

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Conflicts of Interest

The authors declare no conflict of interest.

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