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Proliferative enteropathy (PE; ileitis) is a common intestinal disease affecting susceptible pigs raised under various management systems around the world. Major developments in the understanding of PE and its causative agent, *Lawsonia intracellularis*, have occurred that have led to advances in the detection of this disease and methods to control and prevent it. Diagnostic tools that have improved overall detection and early onset of PE in pigs include various serological and molecular-based assays. Histological tests such as immunohistochemistry continue to be the gold standard in confirming *Lawsonia*-specific lesions in pigs *post mortem*. Despite extreme difficulties in isolating *L. intracellularis*, innovations in the cultivation and the development of pure culture challenge models, have opened doors to better characterization of the pathogenesis of PE through *in vivo* and *in vitro L. intracellularis*–host interactions. Advancements in molecular research such as the genetic sequencing of the entire *Lawsonia* genome have provided ways to identify various immunogens, metabolic pathways and methods for understanding the epidemiology of this organism. The determinations of immunological responsiveness in pigs to virulent and attenuated isolates of *L. intracellularis* and identification of various immunogens have led to progress in vaccine development.

Keywords

Disciplines

Agriculture | Food Microbiology | Immunology of Infectious Disease | Meat Science

Comments

Proliferative enteropathy: a global enteric disease of pigs caused by *Lawsonia intracellularis*

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Abstract

Proliferative enteropathy (PE; ileitis) is a common intestinal disease affecting susceptible pigs raised under various management systems around the world. Major developments in the understanding of PE and its causative agent, *Lawsonia intracellularis*, have occurred that have led to advances in the detection of this disease and methods to control and prevent it. Diagnostic tools that have improved overall detection and early onset of PE in pigs include various serological and molecular-based assays. Histological tests such as immunohisto-chemistry continue to be the gold standard in confirming *Lawsonia*-specific lesions in pigs *post mortem*. Despite extreme difficulties in isolating *L. intracellularis*, innovations in the cultivation and the development of pure culture challenge models, have opened doors to better characterization of the pathogenesis of PE through *in vivo* and *in vitro L. intracellularis*–host interactions. Advancements in molecular research such as the genetic sequencing of the entire *Lawsonia* genome have provided ways to identify various immunogens, metabolic pathways and methods for understanding the epidemiology of this organism. The determinations of immunological responsiveness in pigs to virulent and attenuated isolates of *L. intracellularis* and identification of various immunogens have led to progress in vaccine development.

Keywords: PE, ileitis, *Lawsonia intracellularis*

Introduction

Lawsonia intracellularis is an obligate intracellular bacterium causing proliferative enteropathy (PE) in many mammalian species, most notably pigs. The infection causes diarrhea, stunted growth and, in rare instances, sudden death in pigs and is one of the most economically important diseases in the swine industry worldwide (Lawson and Gebhart, 2000). The disease is characterized by a thickening of the mucosal lining of the small, and sometimes large intestine (Rowland and Lawson, 1992). Other distinguishing features include proliferation of the immature epithelial cells of the intestinal crypts, forming a hyperplastic to adenoma-like mucosa (McOrist and Gebhart, 1999). Histological lesions can be confirmed as *Lawsonia*-specific by visualization of the tiny, vibrioid shaped bacteria found in the enterocytes of the terminal ileum, cecum and spiral colon, and also within macro-phages located in the lamina propria between intestinal crypts, and mesenteric lymph nodes (Frisk and Wagner, 1977; Roberts *et al.*, 1980; McOrist and Gebhart, 1999).

There are several different syndromes of PE. Porcine intestinal adenomatosis (PIA) is considered to be the chronic form of PE mainly affecting young growing pigs (McOrist and Gebhart, 1999). Proliferative hemorrhagic enteropathy (PHE) is often seen in adult pigs and is classified as acute PE resulting in bloody diarrhea, blood

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clots and sudden death (McOrist and Gebhart, 1999). Necrotic enteritis (NE) is less common than PIA and PHE and is found in pigs exhibiting severe thickening of the mucosa with brownish-yellow necrotic lesions on the luminal surface (McOrist and Gebhart, 1999). Recently, a new form of PE has been described in pigs in which *L. intracellularis* infections are subclinical (no sign of disease) and persist in a 'carrier' state (McOrist *et al.*, 2003).

PE is most commonly found in pigs; however, it has also been described in hamsters (Frisk and Wagner, 1977), ferrets (Fox and Lawson, 1988), rabbits (Fox *et al.*, 1994), foxes (Eriksen and Landsverk, 1985), dogs (Collins *et al.*, 1983), rats (Vandenburghe *et al.*, 1985), horses (Williams *et al.*, 1996), sheep (Chalmers *et al.*, 1990), deer (Drolet *et al.*, 1996), emus (LeMarchand *et al.*, 1995), ostriches (Cooper *et al.*, 1997), primates (Klein *et al.*, 1999) and guinea pigs (Elwell *et al.*, 1981). Intestinal lesions are strikingly similar among all the above mentioned species with intracellular bacteria identified as *L. intracellularis* observed in the proliferative epithelia (Lawson and Gebhart, 2000). Despite its ubiquitous nature, *L. intracellularis* has never been identified in humans with enteric disease, even those affected with Crohn's or other related diseases such as colon cancer (McOrist *et al.*, 2003). Therefore, *Lawsonia*-specific PE is not considered to be a zoonotic disease (McOrist *et al.*, 2003).

Etiology

PE has been described as an important enteric disease that has been recognized in pigs for over 70 years. Characteristic lesions of PE found in pigs were first described by Beister and Schwarte in 1931. It was not until the 1970s that intracellular bacteria were found within proliferating crypt cells in cases of PHE in pigs (Rowland *et al.*, 1973). A variety of *Campylobacter* species having morphologically similar features to *L. intracellularis* have been isolated from lesions of PE. Those include *Campylobacter mucosalis* (Rowland and Lawson, 1974; Love *et al.*, 1977), *C. hyointestinalis* (Gebhart *et al.*, 1983), *C. jejuni* and *C. coli* (Erickson *et al.*, 1990). Despite the routine recovery of the above mentioned *Campylobacter* species in proliferative lesions, none of these organisms specifically cause PE or colonize intracellularly under experimental conditions (Kashiwazaki *et al.*, 1971; McCartney *et al.*, 1984; Boosinger *et al.*, 1985; Alderton *et al.*, 1992). It was not until Lawson *et al.* (1985) inoculated rabbits with an extract containing intracellular bacteria from an intestinal lesion that did not contain *Campylobacter* that a new and novel intracellular bacterium was discovered. Convalescent serum containing antibodies from inoculated rabbits did not react to various isolates of *Campylobacter* but reacted to intracellular bacteria in formalin-fixed sections of PE-affected
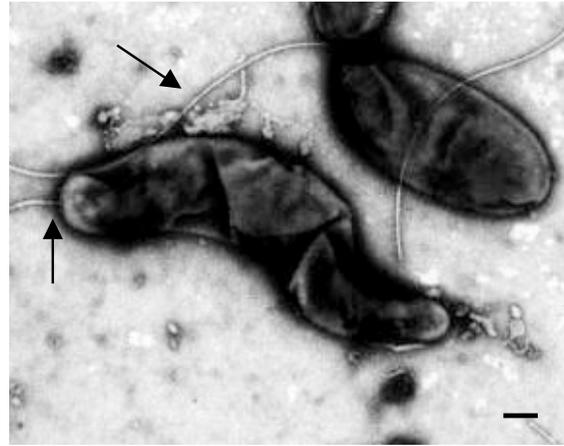

Fig. 1. An electron micrograph of *L. intracellularis* in pure culture. Arrow indicates a single polar flagellum. Bar=10 μm. (Photograph courtesy of Dr. Connie Gebhart.)

intestines (Lawson *et al.*, 1985). Progress in cultivation of this organism ensued and Koch's postulates were fulfilled when pure cultures of the intracellular bacterium were shown to cause PE in pigs (McOrist *et al.*, 1993). Initially, the bacteria were referred to as '*Campylobacter*-like organisms or CLO' because of their similarities in morphology to *Campylobacter* species (McOrist and Gebhart, 1999). Later, the intracellular bacteria were given the name Ileal Symbiont (IS) intracellularis and were identified as a distinct genus that differed from *Campylobacter* species (Gebhart *et al.*, 1993). The name *L. intracellularis* was formally given to the organism in 1995 in honor of the Scottish scientist G. H. K. Lawson as the primary discoverer of the bacterium (McOrist *et al.*, 1995a).

L. intracellularis is a member of the delta division of Proteobacteria (Gebhart *et al.*, 1993) and is taxonomically distinct from other intracellular pathogens (McOrist *et al.*, 1995a). DNA sequences of the 16 S ribosomal RNA gene from *L. intracellularis* were found to be closely related to *Bilophila wadsworthia* (Sapico *et al.*, 1994) and the sulfate-reducing proteobacterium, *Desulfovibrio desulfuricans* (Gebhart *et al.*, 1993), with 92 and 91% homology, respectively. *L. intracellularis* is classified as a Gram-negative, microaerophilic, obligate intracellular, non-flagellated, non-spore-forming, curved or S-shaped bacillus (Lawson *et al.*, 1993), but recently, a long, single, polar flagellum has been observed by electron microscopy in multiple pure culture isolates of *L. intracellularis*; see Fig. 1 (Lawson and Gebhart, 2000). The bacterium measures 1.25–1.75-μm long and 0.25–0.43-μm wide comprising a trilaminar outer envelope separated from the cytoplasmic membrane by an electron-lucent zone; neither fimbriae nor spores have been observed (Lawson and Gebhart, 2000). The entire genome has recently been sequenced at the University of Minnesota (Gebhart and Kapur, 2003) and contains approximately 1.46 Mb.

Isolation and cultivation of *L. intracellularis*

Isolation and cultivation of an obligate intracellular organism is one of the most daunting tasks in bacteriology. *L. intracellularis* is no exception. Currently, the growth of *L. intracellularis* in cell-free media or broth has not been accomplished. Therefore, successful cultivation relies on growth of this bacterium on susceptible eukaryotic tissue culture cells including rat intestinal cells (IEC-18) (Lawson *et al*., 1993), human fetal intestinal cells (Int 407) (Lawson *et al*., 1993), rat colonic adenocarcinoma cells (Lawson *et al*., 1993), pig kidney cells (PK-15) (Lawson *et al*., 1993), piglet intestinal epithelial cells (IPEC-J2) (McOrist *et al*., 1995a), GPC-16 cells (Stills, 1991), and mouse fibroblast cells (McCoy) (Knittel and Roof, 1999). Cultivation techniques include culturing *L. intracellularis* with adherent (Lawson *et al*., 1993; McOrist *et al*., 1995a; Collins *et al*., 1996) or suspension (Knittel and Roof, 1998) tissue culture cells at reduced oxygen atmospheres, preferably an anaerobic environment, at 37°C for 5–7 days post-inoculation. Adherent cultures can be propagated in tissue culture flasks (T-flasks) of various volumes (25–150 cm^2) and require incubation in humidified chambers such as anaerobic gas jars or modified incubators containing 80–90% N_2, 4–10% CO_2 and 0–10% O_2 (Lawson *et al*., 1993; McOrist *et al*., 1995a; Collins *et al*., 1996). In contrast, suspension cultures do not need specialized growth chambers and are propagated in spinner flasks or bioreactors of various sizes (250 ml to 300 l) that regulate the temperature, gas mix, pH, and agitation automatically (Knittel and Roof, 1998). This method (Knittel and Roof, 1998) has allowed the potential growth of large-scale cultures for use in the production of vaccine and diagnostic reagents and has also been used for the attenuation of a Danish isolate of *L. intracellularis* as a potential vaccine candidate (Kroll *et al*., 2004a).

The preferred medium for growing *Lawsonia*-susceptible tissue culture cells is Dulbecco's modified Eagle's medium (DMEM) with bovine serum at concentrations of 5–10% (Lawson *et al*., 1993; Knittel and Roof, 1998; Guedes and Gebhart, 2003b). Tissue cultures are usually infected with 10% v/v of inoculum containing *L. intracellularis* and are monitored daily by taking a representative sample (supernatant containing sloughed or suspended cells and bacteria) and staining them with *Lawsonia*-specific monoclonal antibodies (McOrist *et al*., 1987; Guedes and Gebhart, 2003c) followed by various secondary staining techniques including immunofluorescence (Lawson *et al*., 1993; Knittel *et al*., 1996), immunogold labeling (Collins *et al*., 1996) or immunoperoxidase assays (Lawson *et al*., 1993; Guedes *et al*., 2002a). Cultures are monitored for increases in percent cell infections or for increasing levels of extracellular bacteria and are typically harvested, passed or used for inoculating animals when they reach 80–100% infectivity.

The conventional method for isolating *L. intracellularis* from infected tissue was developed by Lawson *et al*. (1993). This method requires homogenization of infected areas of the intestinal mucosa and subsequent treatment with 1% trypsin in phosphate-buffered saline (0.1 M, pH 7.4). The mucosal homogenates are passed through a series of filters (200-mesh stainless steel, Whatman glass fiber filter, 1.2, 0.8 and 0.65 μm syringe filters) to make a filtrate containing *L. intracellularis* and other intestinal organisms, and then stored in a sucrose potassium glutamate solution with 10% FBS at −70°C (Lawson *et al*., 1993). The filtrates are used to inoculate partially confluent cell monolayers which are incubated with media containing *Lawsonia*-resistant antibiotics that inhibit growth of confounding bacteria and fungi (Lawson *et al*., 1993). Co-cultivation of *L. intracellularis* with tissue culture cells continue until high levels of the bacteria are achieved in pure culture (Lawson *et al*., 1993).

Reproduction of the disease

The development of *in vitro* cultivation methods as described above has provided the means for fulfilling Koch's postulates for PE in pigs. Germ-free pigs developed PE from crude intestinal filtrates containing *L. intracellularis* and other enteric bacteria (McOrist and Lawson, 1989), whereas those exposed to pure cultures of *L. intracellularis* failed to develop disease (McOrist *et al*., 1993). Additionally, gnotobiotic pigs inoculated with *Lawsonia*-containing gut homogenates developed intestinal lesions typical of PE (McOrist *et al*., 1994a). These observations strongly suggest that intestinal flora influences the development of PE by modifying or supporting the ability of *L. intracellularis* to colonize the intestinal tract (Smith and Lawson, 2001). However, the roles of commensal bacterial and other enteric pathogens potentially present during a *Lawsonia* infection are still undefined (Smith and Lawson, 2001).

PE can be reproduced by challenging pigs with *L. intracellularis* using pure culture or intestinal mucosa homogenates from previously infected pigs. The advantages of a pure culture challenge are that an infective dose can be quantified for stringent control of a more defined and consistent reproduction of PE in pigs (Kroll *et al*., 2004a). Pure culture inocula contain no confounding effects due to potentially pathogenic intestinal bacteria or viruses (Guedes and Gebhart, 2003b). The disadvantage is the difficulty involved in the isolation and cultivation of *L. intracellularis in vitro* (Guedes and Gebhart, 2003b). The advantages of an intestinal mucosa homogenate challenge are that it is relatively easy to produce by scraping the *Lawsonia*-infected ileal mucosa from PE-affected pigs and then administering the scrapings immediately to naïve susceptible pigs. Also, this approach has proved to be successful over the years in effectively reproducing all forms of PE in pigs and hamsters (Lomax

et al., 1982a, b; Mapother et al., 1987; McOrist et al., 1993). The disadvantage is that mucosal homogenates contain other microflora and potentially pathogenic organisms (bacteria, viruses and fungi) that confound the effects of a *Lawsonia*-only challenge. Mucosal homogenates are not easily quantified for determining the proper infectious dose and the enumeration methods that are used have not been able to differentiate between live vs. dead *L. intracellularis* organisms (Guedes and Gebhart, 2003b).

A direct comparison of both *L. intracellularis* challenge models was done by Guedes and Gebhart (2002c, 2003b) who demonstrated that reproduction of clinical signs and lesions typical of PE was similar in both models.

Gut homogenate challenge model

Experiments designed to reproduce PE in pigs were only successful when the orally administered inocula contained *L. intracellularis* in fresh mucosal homogenates derived directly from affected pigs (Roberts et al., 1977; Lomax et al., 1982a, b; Mapother et al., 1987; McOrist and Lawson, 1989). This model is commonly used because it can be rapidly produced with minimal effort and little technical skill prior to challenge. Until recently, gut homogenate or mucosa-derived *Lawsonia* challenges had a history of providing a severe challenge in pigs that may not have correlated to typical PE found in nature. Guedes et al. (2003a) reported the use of a refined gut homogenate challenge model using lower concentrations of semi-quantified *L. intracellularis* in the challenge inoculum. In this study, 10-fold dilutions of a highly virulent gut homogenate challenge resulted in disease reproduction that closely resembled field outbreaks. The lowest dose administered (approximately 5.4×10^8 *Lawsonia* dose^{-1}) produced infection in susceptible pigs, indicating that a low infectious dose is sufficient to reproduce field-type clinical symptoms of PE. These results were consistent with previous studies in which pigs challenged with approximately 10^7–10^8 *Lawsonia* organisms per dose developed moderate to severe diarrhea beginning 2 weeks post-inoculation (Collins et al., 2001). To date, a minimum infectious dose has not been determined.

Pure culture challenge model

The *L. intracellularis* pure culture challenge model was originally developed by McOrist et al. (1993) in order to demonstrate reproduction of PE in pigs that were orally inoculated with *Lawsonia*-infected enterocytes. In this study, four pigs that were challenged with approximately 10^6 *Lawsonia* organisms per dose developed subclinical PE. There were no clinical signs, but the animals had

gross and microscopic (IHC) lesions consistent with the presence of *L. intracellularis*.

Gross and IHC lesions typical of acute PE were induced in pigs that were given a pure culture of *L. intracellularis* to show that stress induced by dexamethasone had no effect on the development of intestinal lesions (Joens et al., 1997). A pure culture of *L. intracellularis* isolate N343 was successfully used in multiple controlled challenge exposure studies for determining the efficacy of Denagard^{TM} (tiamulin hydrogen fumarate) when given orally to pigs in the feed or drinking water (McOrist et al., 1996b; Schwartz et al., 1999; Walter et al., 2000a, b). Additionally, virulent *L. intracellularis* pure culture challenge exposure studies have been performed to establish vaccine efficacy against PE (Kroll et al., 2004a). Uses of pure culture *L. intracellularis* challenge models have provided valuable information on the transmission of the organism within pig herds (Smith and McOrist, 1997; Jordan et al., 2004). This model is used in limited fashion because of the extreme difficulties in the isolation and cultivation of *L. intracellularis* in the laboratory.

Pathogenesis

Exposing susceptible pigs to *L. intracellularis* or to diseased mucosa containing these intracellular bacteria can reproduce PE. In typical oral challenge exposure studies of weaned conventional pigs (4 weeks old) with a standard inoculum of 10^8 *L. intracellularis* bacteria, numerous intracellular bacteria can be visualized in the developing proliferative intestines and in the feces 1–3 weeks following inoculation, with a peak of infection and lesions 3–4 weeks after challenge (McOrist et al., 1996a). In most of these pigs, intestinal infection, proliferative lesions and excretion persist for approximately 4 weeks, but in some exposed pigs, excretion may persist for at least 10 weeks (Smith and McOrist, 1997). At the peak of infection, moderate diarrhea and histological lesions of PE are usually observed in 50 and 100%, respectively, of animals challenged with this inoculum (McOrist et al., 1996a, 1997b). Infection and lesions in the large intestine (colon and cecum) generally start to occur a week or two after small intestinal infection, following an oral challenge (McOrist et al., 1996a). Naïve pigs of a wide age range (neonates to grower-finishers) are susceptible to oral challenge (McOrist and Gebhart, 1999).

PE initially develops as a progressive proliferation of immature epithelial cells, following invasion of the intracellular *Lawsonia* bacteria. In most cases, no significant inflammatory reaction occurs and the bacteria remain in the epithelium at this stage (McOrist et al., 1996a). In severe cases of PE, *L. intracellularis* can also be observed in the mesenteric lymph node and tonsils, but these appear to be secondary sites of infection (Jensen et al., 2000). *In vivo* and *in vitro* studies have elucidated

some of the early events in bacteria–cell interaction (Lawson *et al*., 1993; McOrist *et al*., 1995c). Bacteria associate closely with the cell membrane and then quickly enter the enterocytes via an entry vacuole (McOrist *et al*., 1995c). Specific adhesins or receptors have not been identified but attachment and entry appear to require specific bacterium–host cell interaction (McOrist *et al*., 1997c). The genome sequence of *L. intracellularis* indicates that it may possess a type III secretion system. This secretion system, commonly found in Gram-negative bacterial pathogens, may assist the bacterium during cell invasion and evasion of the host's immune system and could be a mechanism for inducing cellular proliferation.

The entry of *Lawsonia* bacteria into cells is dependent on host cell activity, but not necessarily bacterial viability, possibly indicating a type of induced phagocytosis like receptor-mediated endocytosis (Lawson *et al*., 1993). Actin rearrangement is important for entry of obligate intracellular bacteria and may be a key component of entry for *L. intracellularis* (Lawson *et al*., 1995). *Lawsonia* gene sequence analysis has revealed several genes that are involved in producing a single polar flagellum which may also play a role in entry of the host cell (Nuntaprasert *et al*., 2004). Morphological associations of *L. intracellularis* and small pits or vesicles of the cell membrane were observed immediately upon entry (McOrist *et al*., 1995c). These events are similar to the association between *Chlamydia trachomatis* and *C. psittaci* entry and clatherin-coated pits (Reynolds and Pearce, 1991).

Other factors may be involved as experiments using cytochalasin D (blocks cytoskeleton rearrangements) indicate that there may also be a non-actin-dependent pathway utilized by *L. intracellularis* for successful host cell invasion (Lawson *et al*., 1995). The entry vacuole rapidly breaks down (within 3 h) and *L. intracellularis* flourish and multiply free (not membrane-bound) within the apical cytoplasm (McOrist *et al*., 1995c). This mechanism would explain how *L. intracellularis* escapes proteolytic degradation due to endosome–lysosome fusion. Many other species of intracellular bacteria including *Shigella*, *Listeria*, and some *Rickettsia* species also escape into the cytoplasm and avoid the damaging effects of phagolysosomal fusion by producing membrane-damaging cytolytic enzymes or toxins such as phospholipase or listeriolysin (Ewing *et al*., 1978; Todd *et al*., 1981; Gaillard *et al*., 1987; Sansonetti, 1992). *L. intracellularis* exhibits cytolytic (hemolytic) activity *in vitro* through expression of a novel hemolysin, *Lawsonia* hemolysin A (LhyA), which may be one of the main virulence factors involved in intracellular vacuole escape (Smith, 2001).

Following vacuolar escape, the bacterial–host cell relationship observed *in vitro* is similar to that found in animals (McOrist *et al*., 1995c). Typically, *Lawsonia* bacteria located in the apical cytoplasm do not localize to any cell structures except for some association with the cell mitochondria (McOrist *et al*., 1995c) and the rough endoplasmic reticulum (Jansi *et al*., 1994).

Intracellular multiplication and cell to cell spread of *L. intracellularis* were revealed when co-cultivation experiments demonstrated that cells infected with bacteria continued to divide and spread the bacteria into newly developed daughter cells (Lawson *et al*., 1993). Additional evidence suggests that actively dividing cells promote bacterial propagation better than non-dividing, mature cells (Lawson *et al*., 1993; McOrist *et al*., 1995c). Experiments using cycloheximide or colchicine to stop eukaryotic cell division also inhibited *L. intracellularis* growth (Lawson *et al*., 1995). Furthermore, growth promotion of *L. intracellularis* was better in rapidly growing enterocyte cultures than in confluent monolayers (Lawson *et al*., 1993). Dividing enterocytes benefit *Lawsonia* proliferation by facilitating bacterial expansion through continued replication and migration, and mediate spread of the bacteria throughout the epithelium (Smith and Lawson, 2001).

Cell proliferation in PE involves cells infected by *L. intracellularis* and, where islands of hyperplasia occur amidst normal epithelium, only infected cells are proliferative (McOrist *et al*., 1996a). Infection experiments in hamsters indicate that crypt cells start to divide at an increased rate (up to 4-fold) 2 days after bacterial infection (Jansi *et al*., 1994). The stimulatory effect of the bacteria on cell division does not persist once the lesion becomes fully developed despite the constant presence of *L. intracellularis* (McOrist *et al*., 1996a). The mechanism whereby *L. intracellularis* prevents cell maturation is not known, but cells continue to undergo mitosis and proliferation, and form hyperplastic crypts (McOrist *et al*., 1996a). *L. intracellularis*-infected intestinal crypts can become enormously elongated and often branched (McOrist and Gebhart, 1999). Loss of protein and amino acids into the intestinal lumen and reduced nutrient absorption by the intestinal mucosa are the likely causes of the reduction in weight gain and feed conversion efficiency seen in pigs affected with chronic uncomplicated proliferative enteropathy lesions (McOrist and Gebhart, 1999).

Clinical signs

Clinical cases of PIA are observed most commonly in the post-weaned pigs between 6 and 20 weeks of age (McOrist and Gebhart, 1999). The predominant signs of PIA include anorexia, diarrhea, and poor growth which persist for a period of weeks (Lawson and Gebhart, 2000). Diarrhea may occur when significant lesions are present (McOrist and Gebhart, 1999), which makes this form of PE very difficult to detect. In PIA endemic herds, pigs will exhibit normal feed intake but will fail to sustain normal growth (McOrist and Gebhart, 1999). Severely infected pigs are often associated with varying degrees

Fig. 2. Above: a gross lesion containing fibrinous blood clots indicative of PHE caused by *L. intracellularis*. Below: the normal appearance of an ileum from a healthy pig. (Photograph courtesy of Dr Jeff Knittel.)

of thickening in the mucosal lining or necrotic lesions of the small intestine commonly described as 'hose pipe or garden hose' gut (Rowland and Lawson, 1992).

Cases of PHE occur more commonly in young adult pigs between the ages of 4 and 12 months (McOrist and Gebhart, 1999). Black tarry feces are the first visible clinical sign commonly followed by a loose, red-tinged, watery stool (McOrist and Gebhart, 1999). However, some of the pigs die without fecal abnormality (McOrist and Gebhart, 1999). It has been estimated that half of the pigs affected with PHE will die and the other half recover over a short period of time without visible signs of reduced weight or body condition (Rowland and Lawson, 1992).

Subclinical PE may be the most common disease among growing pigs, but the condition is rarely recognized because there are no observable clinical indications in pigs with *Lawsonia*-specific subclinical enteritis. This variation of PE closely resembling PIA is defined as active *L. intracellularis* infection with the presence of microscopic and/or gross lesions resulting in reduced productivity (average daily gain with +/− feed efficiency) in the absence of observable clinical signs of disease such as PE-associated mortality, diarrhea or other symptoms consistent with PE morbidity. Evidence of a subclinical *L. intracellularis* infection may or may not be detected by serological or polymerase chain reaction (PCR) methods as pigs may be colonized but not severely enough to induce shedding or seroconversion (Guedes, 2004).

There is a variety of enteric diseases that display similar clinical symptoms to the various forms of PE. These include hemolytic bowel syndrome (HBS), colibacillosis, porcine circovirus 2 (PCV2) diseases, transmissible gastroenteritis (TGE), rotavirus infection, salmonellosis, and swine dysentery. It is therefore important to differentiate among all of the common factors by performing a thorough post mortem examination and implementing proper diagnostic evaluations.

Lesions

Histopathological lesions common to all forms of PE are characterized by the adenomatous proliferation of the epithelium in the crypts of the small intestine and in mucosal glands of the large intestine, and by the presence of curved intracellular bacteria within these enterocytes (Rowland and Lawson, 1974). The crypts are elongated, enlarged and lined with crowded immature epithelial cells with mitotic events (McOrist and Gebhart, 1999). Goblet cells are absent from the affected epithelium and the infiltration of inflammatory cells is not a common characteristic of PE (Rowland and Lawson, 1992). The proliferating epithelial cells contain intracytoplasmic, slender, curved, rod-shaped bacteria (Ward and Winkelman, 1990).

PHE

The acute and most severe form of PE is considered to be PHE and typically affects the terminal ileum and colon. This form of PE is most often associated with young adult pigs 4–12 months old (McOrist and Gebhart, 1999) and is commonly found in high health herds when replacement gilts and boars have been introduced into a new farm site. Pathological lesions of PHE include extended and thickened intestines with serosal edema and a severely proliferated mucosa (McOrist and Gebhart, 1999). The lumen contains either fresh blood or a solid cast of blood and fibrin clots as seen in Fig. 2 (Ward and Winkelman, 1990). However, focal points of bleeding, ulcerations or erosions are not observed (McOrist and Gebhart, 1999).

Fig. 3. A gross lesion containing mild to moderate thickening of the intestinal mucosa indicative of PIA caused by *L. intracellularis.*

Fig. 4. A gross lesion containing severe thickening and hemorrhaging of the intestinal mucosa, necrotic ulcerations and evidence of a fibrinous cast indicative of NE caused by *L. intracellularis.*

PHE is differentiated from HBS, in which there is no abnormal crypt proliferation and the hemorrhage occurs throughout all layers of the intestinal wall (Knittel, 1999).

PIA

The chronic and most common form of PE is considered to be PIA. This form of PE is commonly found in actively growing pigs from late nursery to late finishing stages and affects the terminal 50 cm of ileum and the upper third of the proximal colon (McOrist and Gebhart, 1999). Pathological lesions of PIA consist of intestinal mucosal thickening by epithelial proliferation but relatively free from inflammation or only mildly inflamed on the mucosal surface as seen in Fig. 3 (Knittel, 1999). Histologically, the mucosa is enlarged with branching

crypts lined with immature epithelial cells (McOrist and Gebhart, 1999). Mitotic figures are evident throughout the crypt while goblet cells are non-existent (McOrist and Gebhart, 1999). Intracellular bacteria are a common feature in the apical cytoplasm of the affected epithelial cells (McOrist and Gebhart, 1999).

NE

Considered a result of end-stage PIA, NE involves deep coagulative necrosis of the adenomatous mucosa (Rowland, 1978). Yellowish-gray lesions are evident on the surface of the mucosal lining in the terminal portion of the ileum (Rowland and Lawson, 1992) (Fig. 4). Severe thickening of the ileum in these cases have given the disease a unique characteristic of PE called

Table 1. Summary of diagnostic techniques for detection of *L. intracellularis* and/or antibodies to the organism in pigs

Diagnostic technique	Detection method	Advantages	Disadvantages	Reference
H&E	Crypt hyperplasia, cell abnormalities	Confirms cell abnormalities with bacterial involvement in tissues	Post mortem diagnosis only	Rowland (1978)
WS silver stain	Identification of intracellular organisms	Rapid detection of bacterial involvement in tissues	• Non-specific • Post mortem diagnosis only	Ward and Winkelman (1990) Rowland and Lawson (1992) Jensen et al. (1997)
IHC/IFA	*Lawsonia*-specific immunoflourescence or immunoperoxidase staining	Highly sensitive and specific	• Post mortem diagnosis only • Requires a monoclonal antibody	McOrist et al. (1987) Knittel et al. (1997) Guedes and Gebhart (2003c)
PCR	*Lawsonia*-specific sequences	• Ante mortem diagnosis • Highly specific • Detects active colonization and shedding • Detection of multiple pathogens in one test • Real-time detection	• Less sensitive • Possible inactivation of test reagents • Possible cross-contamination of samples • Possibility of false negatives	Gebhart et al. (1991, 1993) Jones et al. (1993a, c) Cooper (1996), Cooper et al. (1997) Elder et al. (1997) Lindecrona et al. (2002)
IFAT	Serum IgG	• Ante mortem diagnosis • Highly sensitive and specific	Requires manual determination of results	Knittel et al. (1998)
IPMA	Serum IgG	• Ante mortem diagnosis • Highly sensitive and specific	Requires manual determination of results	Guedes et al. (2002a)
LPS-ELISA	Serum IgG	• Ante mortem diagnosis • Highly sensitive and specific • Automated determination of results • Rapid detection • High throughput testing	• Possible batch-to-batch variation • Possible lab to lab variation • Not commercially available	Kroll et al. (2005b) Boesen et al. (2005b)

'hose pipe or garden hose' gut (Rowland and Lawson, 1992).

Diagnosis

For many years, diagnosis of PE in pigs was speculative as clinical symptoms such as diarrhea or gross and microscopic examination of the intestines were the only way to determine if pigs were affected with the disease. With the advent of sensitive and specific diagnostic methods, new strides have been made to identify *L. intracellularis*-specific infections and the prevalence of PE in pig herds. These methods have assisted veterinarians and pig producers in determining with better precision when to implement control and prevention measures in herds in which PE is endemic. A summary of the various diagnostic techniques available for detecting *L. intracellularis* exposure in animals is presented in Table 1.

Histolopathology and immunohistochemistry

PE is often diagnosed *post mortem* by the characteristic gross pathology associated with *L. intracellularis* infections. However, confirmation of PE through histopathological analysis is necessary in order to ensure proper diagnosis. Hematoxylin and eosin (H&E) staining of tissue sections exhibiting severe PE identifies proliferative changes in the enterocytes of the intestines (Rowland, 1978). Warthin Starry (WS) silver stain allows the detection of curved, rod-shaped, intracellular bacteria in histological sections, but this staining technique is non-specific and has limitations when applied to necrotic or autolyzed samples (Ward and Winkelman, 1990; Rowland and Lawson, 1992; Jensen et al., 1997). A modified Ziehl–Neelsen stain on mucosal and fecal smears or fixed infected eukaryotic cell samples of *L. intracellularis* provides a non-specific, simple confirmatory test as the intracellular bacteria stain red within

the cytoplasm of infected cells (Ward and Winkelman, 1990; Rowland and Lawson, 1992). Immunohisto-chemistry (IHC) and immunofluorescence antibody (IFA) procedures have made it possible for specific detection of *L. intracellularis* within tissue sections affected with various forms of PE (Knittel *et al*., 1997). With the use of *Lawsonia*-specific monoclonal antibodies, the bacteria can be identified in fecal smears of cases involving high levels of actively shedding *L. intracellularis* or in fixed tissue sections from various sources (McOrist *et al*., 1987; Guedes and Gebhart 2003c). A comparative study revealed that the IHC (86.8%) stain was much more sensitive than H&E (36.8%) and WS (50%) staining methods for detecting *L. intracellularis* in PE-affected tissue samples (Guedes *et al*., 2002b). In addition, there was a high correlation (82.5%) between the IHC results and the presence of macroscopic lesions 4 weeks after infection (Guedes *et al*., 2002b). These results are consistent with previous reports that a histological staining technique involving the use of an anti-*Lawsonia* monoclonal antibody was highly specific and more sensitive in detecting *L. intracellularis* infections than non-specific histological stains (McOrist *et al*., 1987; Jones *et al*., 1993b; Huerta *et al*., 2003). Using IHC, *L. intracellularis* positive antigen can be detected even in cases of severe necrosis in which the mucosa is completely destroyed, or during recovery stage when the bacterial antigen is found only in the cytoplasm of mononuclear cells in the lamina propria (Guedes *et al*., 2002b).

Serology

Early accounts of the first serology test for detecting *Lawsonia*-specific IgM and IgA antibodies in naturally exposed or experimentally infected pigs were from Lawson *et al*. (1988). In these studies, an indirect fluorescent antibody test (IFAT) was used to determine that IgM and, to a lesser extent, IgA was the predominant antibody in early *L. intracellularis* infections in pigs lasting about 8 weeks post-inoculation. Holyoake *et al*. (1994) employed an enzyme-linked immunosorbent assay (ELISA) using Percoll-purified whole cell *L. intra-cellularis* from PHE-affected pig tissue as the primary antigen in the test system. This ELISA detected *Lawsonia*-specific IgG in field-exposed pigs as early as 3 weeks of age, and represented the first recorded detection of maternally acquired antibodies. However, the antibody titers were low and variable among inoculated and uninoculated test groups and could not be differentiated statistically (Holyoake *et al*., 1994).

An IFA test modified to detect IgG instead of IgM or IgA was developed using pure culture derived *L. intra-cellularis* (Knittel *et al*., 1998). This assay incorporated the use of *Lawsonia* antigen grown in pure culture that is then stained using serum from test pigs as the primary

antibody and fluorescein isothiocyanate (FITC)-labeled anti-swine IgG (heavy and light chains) as the secondary antibody (Knittel *et al*., 1998). Results from three experiments indicated that the IFAT was more sensitive (90%) and highly specific (96%) compared to PCR (47 and 100%, respectively) in detecting *L. intracellularis* anti-bodies 21–28 days after inoculation of pigs challenged with pure culture (Knittel *et al*., 1998). Maternal anti-bodies were detected by the IFAT for at least 5–6 weeks of age in these evaluations. The IFAT has proven to be an accurate and reliable *ante mortem* tool for detecting the incidence of *L. intracellularis* in experimental and naturally exposed pigs around the world (Bakker *et al*., 2000; Dunser *et al*., 2000; Fourchon and Chouet, 2000; Mortimer *et al*., 2000; Just *et al*., 2001; Guedes *et al*., 2002b).

An immunoperoxidase monolayer antibody assay (IPMA) was developed to provide an alternative to the immunoflourescence-based assay for detecting serocon-version to *L. intracellularis* (Guedes *et al*., 2002a). This assay is very similar in design and utility as the IFAT except for the following: the secondary antibody is an anti-swine IgG peroxidase-labeled conjugate (vs. FITC), the methods by which sample preparations are observed are different (IPMA=light microscopy; IFAT=UV micro-scopy), and the IPMA requires one more step than the IFAT (incubation with H_2O_2 to remove endogenous peroxidase) (Guedes *et al*., 2002a). The sensitivity (88.9%) and specificity (100%) of this assay in detecting anti-*Lawsonia* antibodies in pigs were comparable to the IFAT (Guedes *et al*., 2003b). Both assays were compared in parallel in a controlled challenge exposure study in which serum was tested on days 7, 14, 21 and 28 after oral inoculation with intestinal homogenate from PHE-infected pigs (Guedes *et al*., 2003b). Results showed that the percentage of agreement between the IFAT and IPMA was 98.6% (Guedes *et al*., 2003b). The serology results agreed in all samples tested except on days 0 and 7 in which one control animal (*Lawsonia*-negative) was positive by the IPMA, but negative by IFAT (Guedes *et al*., 2003b). These results from controlled infection studies suggest that either assay can be used to effectively detect *L. intracellularis* exposure in pigs. Regardless of which test is used to detect PE in pigs, serological results should be carefully interpreted as antibody detection may vary due to several factors including natural vs. challenge controlled *L. intracellularis* infections, individual pig vs. whole herd screening or lab to lab variation in the interpretation of test results. Corzo *et al*. (2005) revealed only a fair agreement (kappa coefficient (κ)=0.28) between IFAT and IPMA test results from pigs naturally exposed to *L. intracellularis* on an individual (pig to pig) level. Results from these studies showed better agreement (κ=0.55) in interpretation at the herd level (pig groups) (Corzo *et al*., 2005).

Various authors have attempted to develop ELISA methods that would be as reliable in detecting

anti-*Lawsonia* antibodies in sera as the previously described assays. An ELISA would be the desired test method for detecting exposure to *L. intracellularis* in pigs because it allows for high throughput sample testing, automated reporting of test results and an unbiased determination of a *Lawsonia*-positive or -negative sample. An ELISA (Watarai *et al.*, 2004) was developed using synthetically produced peptides of the *L. intracellularis* outer surface antigen, LsaA (McCluskey *et al.*, 2002). This ELISA was able to distinguish between rabbits naturally infected with *L. intracellularis* from those that were *Lawsonia*-naïve (Watarai *et al.*, 2004). These results indicated that a single antigenic molecule may be used as the primary antigen in an indirect ELISA format for successfully detecting anti-*Lawsonia* IgG in rabbit serum (Watarai *et al.*, 2004). Recently, an ELISA was developed for experimentally detecting anti-*Lawsonia* IgG in pigs that were previously inoculated with virulent pure culture *L. intracellularis* (Kroll *et al.*, 2005b). In this test system, an indirect ELISA format using the lipopolysaccharide (LPS) component of a virulent *L. intracellularis* pure culture isolate as the primary antigen was successful in detecting anti-*Lawsonia* IgG antibodies in pigs. Compared to the IFAT, the LPS-based ELISA detected significantly (*P*<0.05) more anti-*Lawsonia* IgG positive pigs after vaccination and challenge on days 21, 28, 35 and 42 of the study (Kroll *et al.*, 2005b). The sensitivity (99.5%) and specificity (100%) of this assay were slightly higher than those of the IFAT, suggesting that this test method may be better at detecting early onset of *L. intracellularis* exposure in pigs regardless of isolate type (vaccine or wild-type) (Kroll *et al.*, 2005b).

Others have used similar technology in a capture-antibody or sandwich ELISA format in which *L. intra-cellularis* LPS was immobilized with a monoclonal antibody to the bottom of each well in a microtiter plate (Boesen *et al.*, 2005a, b). In these studies, 36 of 37 (97%) experimentally infected pigs tested positive, while 31 of 31 (100%) weaned pigs 12 weeks of age and 30 of 31 (97%) weaned pigs 20 weeks of age tested positive for anti-*Lawsonia* IgG antibodies in naturally infected herds. The reported specificity (99.3%) and sensitivity (98%) of this ELISA are similar to those of the indirect LPS-based ELISA and higher than those of the IFAT and IPMA assays (Boesen *et al.*, 2005b). The percentage of agreement between the IFAT was found to be 98.8%, which is nearly the same as the agreement found between the IFAT and IPMA (98.6%) (Boesen *et al.*, 2005b).

PCR

PCR is a sensitive, molecular-based DNA detection tool that can detect low levels of microbial pathogens, especially intracellular organisms. PCR is particularly valuable for use with organisms that may be difficult to recover using conventional isolation methods and/or

detect by microscopy or immunodiagnostic tests. This highly sensitive, pathogen-specific and rapid method has been used successfully for detection of *L. intracellularis* in feces and mucosal samples (Gebhart *et al.*, 1993; Jones *et al.*, 1993a, d) as well as from various tissues including ileum, cecum, colon (Jones *et al.*, 1993b), tonsil (Jensen and Svensmark, 2000), liver and lymph nodes (Jensen *et al.*, 2000). Primers were designed to target specific unique sequences of DNA based on 16 S rDNA found to be conserved among all *L. intracellularis* organisms (Gebhart *et al.*, 1991, 1993; Jones *et al.*, 1993a, c; Cooper, 1996; Cooper *et al.*, 1997). Two sets of primers, an external primer set that amplifies a 319 base pair (bp) fragment of DNA and an internal primer set that amplifies a 255-bp fragment, have been used in nested PCR assays for detecting as little as 10^3 *L. intracellularis* organisms within a gram of feces (Jones *et al.*, 1993b). This nested PCR method was optimized by enhancing procedures for extracting genomic DNA from pig feces, thus achieving a detection limit of 2×10^2 *L. intracellularis* bacteria per g of feces (Moller *et al.*, 1998b). Still, others have found the sensitivity of this assay to be approximately 10^1 *L. intracellularis* bacteria per g of feces (Chang *et al.*, 1997).

Several authors have evaluated PCR for detection of *L. intracellularis* in the feces of experimentally challenged and field exposed pigs (Jones *et al.*, 1993b; McOrist *et al.*, 1994b; Knittel *et al.*, 1998). McOrist *et al.* (1994b) suggested that PCR will only detect positives in feces when pigs have active lesions and *L. intracellularis* is present in high numbers. However, PCR on pig feces has been used to demonstrate that pigs shed *L. intracellularis* in the presence or absence of clinical signs or gross lesions of PE (Knittel *et al.*, 1997; Jordan *et al.*, 1999, 2004). The PCR method has been a useful diagnostic tool to identify the prevalence of *L. intracellularis* in many different pig production systems around the world (Dunser *et al.*, 2000; Keita *et al.*, 2004; Lofstedt *et al.*, 2004; Moller *et al.*, 1998a; Plawinska *et al.*, 2004; Suto *et al.*, 2004; Tomanova and Smola, 2004; Vestergaard *et al.*, 2004; Wendt *et al.*, 2004).

PCR can be used to identify pigs that are actively shedding the organism, but it cannot detect *Lawsonia*-colonized pigs that are not shedding the organism (Jordan *et al.*, 1999). Due to intermittent shedding commonly found in subclinical or chronically infected pigs, false negative results can occur when using PCR on pig feces (Knittel *et al.*, 1997; Jordan *et al.*, 1999). Factors which are naturally found in feces and might inhibit successful PCR, thereby contributing to false negative results, are molecules that inactivate DNA polymerase, degrade or capture nucleic acids, or interfere with cell lysis during the extraction process (Lantz *et al.*, 2000; Jacobson *et al.*, 2003a). The use of an internal control or 'mimic' DNA molecule that is amplified by the same primers as those used for *Lawsonia*-specific DNA would validate the PCR reaction and indicate false-negative

results in clinical specimens (Jacobson *et al.*, 2003a). Jacobson *et al.* (2003a) successfully developed a mimic molecule consisting of a human β-actin molecule that upon amplification results in a larger size (596-bp) DNA fragment than the *Lawsonia*-specific amplicon (319-bp). Due to the size of fragment and the source of mimic DNA (human), this mimic is expected not to competitively exclude the target DNA or non-specifically bind to DNA of bacterial or pig origin. In a recent study, the sensitivity was 10^1–10^2 mimic molecules per reaction tube in *Lawsonia*-spiked tissue samples, and 10^2–10^3 mimic molecules per reaction tube in fecal samples (Jacobson *et al.*, 2004). Still, some PCR inhibitors may exist and interfere with the DNA polymerase since the results improved with the use of enzymes (i.e. rTth and Tli polymerases) known to be less sensitive to inhibition (Al-Soud and Radstrom, 2001).

Multiplex PCR assays were developed for simultaneous detection and identification of *Serpulina* (*Brachyspira*) *hyodysenteriae*, *Salmonella* sp. and *L. intracellularis* in pig feces (Elder *et al.*, 1997; La *et al.*, 2004; Zmudzki *et al.*, 2004). A one-step PCR assay was developed to detect a *Lawsonia*-specific 210-bp DNA fragment in clinical specimens (Suh *et al.*, 2000). This method was found to be highly specific in detecting *Lawsonia*-only DNA in crude intestinal samples (no reaction to swine genomic DNA and other enteric bacterial pathogens) and found to be more sensitive than conventional PCR (Suh *et al.*, 2000).

Researchers have developed a 5′ nuclease assay in which the PCR product amplified by two specific primers based on the 16 S rRNA gene of *L. intracellularis* was detected by fluorescence (Lindecrona *et al.*, 2002). Out of 204 clinical samples, 111 (54%) samples tested positive for *Lawsonia*-specific DNA compared to 98 (48%) samples by IHC making it just as sensitive as IHC in detecting *L. intracellularis* in pig feces (Lindecrona *et al.*, 2002). The detection limits were determined to be approximately 4×10^4 *L. intracellularis* bacteria per g of feces (Lindecrona *et al.*, 2002). Others have designed a real-time PCR method for rapid detection and quantification of *Lawsonia* DNA in high throughput situations (Beckler *et al.*, 2003). Of the 45 known positive samples, 31 were PCR positive by conventional PCR and 36 were positive by real-time PCR, showing sensitivity values of 69 and 80%, respectively (Beckler *et al.*, 2003). Real-time PCR methods like those mentioned above are more attractive than conventional PCR because they allow for immediate confirmation of *Lawsonia*-positive samples and provide the option for quantification, which may be done with pure cultures, mucosal homogenates or feces. In addition, real-time PCR eliminates the need to visualize PCR products via gel electrophoresis leading to a reduction of time, labor and potential problems with cross-contamination. More studies are necessary to optimize real-time PCR technology for the detection and quantification of *L. intracellularis* in pigs.

Other diagnostic techniques

Prior to the development of PCR, a DNA hybridization technique with an *L. intracellularis*-specific DNA probe was used to detect *Lawsonia*-specific genomic DNA in the feces of experimentally infected pigs (Jones *et al.*, 1993d). DNA was extracted from fecal samples and bound to a nylon membrane, then probed with a digoxigenin-labeled *Lawsonia* DNA probe (Gebhart *et al.*, 1991). Another hybridization technique using *Lawsonia* DNA hybridized *in situ* to tissue samples taken from PHE and PIA cases confirmed sequence similarities in *L. intracellularis* from both forms of PE (Gebhart *et al.*, 1991).

An enzyme-linked oligosorbent assay (ELOSA) was developed to specifically identify a 328-bp PCR-amplified *L. intracellularis* DNA fragment in clinical samples (Zhang *et al.*, 2000). Positive test results involved a signal greater than or equal to an optical density of 0.375 at 450 nm wavelength after hybridization of biotin-labeled PCR products with an amine-modified *Lawsonia*-specific internal oligonucleotide capture probe immobilized in 96-well microtiter plates forming an avidin–biotin–peroxidase complex (Zhang *et al.*, 2000).

An immunological method using immunomagnetic separation and ATP bioluminescence was developed for the detection of *L. intracellularis* in fecal samples (Watarai *et al.*, 2005). Magnetic beads coated with an anti-*Lawsonia* LsaA antibody were used to capture whole cell *L. intracellularis* in fecal samples from infected rabbits. The beads containing captured *L. intracellularis* were treated to release ATP and assayed to determine the amount of ATP in each sample. Results from these experiments revealed ATP concentrations higher for anti-LsaA antibody-coated magnetic beads exposed to fecal samples from infected rabbits than those exposed to fecal samples from uninfected rabbits (Watarai *et al.*, 2005). This method could be useful as an alternative to PCR for the detection of active *L. intracellularis* infections in animals.

Epidemiology and economics

PE is an endemic disease that is widespread among swine herds and production systems across every continent where pork production can be found. Previous estimates indicated that PE in growing pigs resulted in direct financial losses of $3–11 US dollars (USD) per pig in the United Kingdom (McOrist *et al.*, 1997a). In the late 1980s and 1990s, annual costs to global pig production were estimated at $20 million USD in the United States (Mapother *et al.*, 1987), $3–6.5 million USD in the United Kingdom (McOrist *et al.*, 1997a) and $25 dollars per sow in Australia (Cutler and Gardner, 1989). However, these estimates reflect pigs having clinical symptoms of

PE or growing pigs with chronically affected PIA (McOrist, 2005). This leads to an underestimation of the overall economic impact of PE that includes clinical (chronic and acute PE) and subclinical (presence of intestinal lesions but without clear diarrhea or weight loss) cases in adult and growing pigs (McOrist, 2005). Most recent estimated losses exceed €100 ($121 USD) per affected breeding pig which translates to an extra €0.50 ($0.61 USD) per growing pig (McOrist, 2005). The total losses due to endemic PE on most European farms could potentially exceed €1 ($1.22 USD) per affected grower-finisher pig (McOrist, 2005).

Many factors contribute to the prevalence and economic impact of *L. intracellularis* on pig farms including age, breed, diet, herd health status, use of antibiotic, vaccines, disinfectants and management and production systems. Other contributing factors having strong influence in maintaining PE in pig herds are the transmission and survival of *L. intracellularis* in the environment. However, the understanding of the true nature of the epidemiology and economics of PE on pig farms is largely contingent upon the availability and extensive use of more refined and reliable *ante mortem* diagnostic assays. Rates of exposure or prevalence of *L. intracellularis* in swine herds have been determined through serological techniques such as IFAT (Knittel *et al.*, 1998) and the IPMA (Guedes *et al.*, 2002a). PCR (Jones *et al.*, 1993a) methodology has proven to be a reliable molecular diagnostic tool for specifically detecting *Lawsonia* DNA in pig feces. Serology can provide historical information on exposure to the bacteria, while PCR tests are measures of actual infection (Guedes, 2004).

Another option for tracking *L. intracellularis* isolates from different geographical origins is a PCR-based molecular tool that detects variable number tandem repeats (VNTR) within the *Lawsonia* genome (Beckler *et al.*, 2004). Gebhart and colleagues have demonstrated that this assay can be used to screen *Lawsonia*-infected tissues and can discriminate between isolates based on the number of highly variable tandem repeats among four loci (Beckler *et al.*, 2004). High discrimination of isolates into genotypic subtypes was possible between outbreaks of PE on geographically distinct pig farms and between animal species including pigs, horses, hamsters, ferrets, ostrich and spider monkey (Weber *et al.*, 2004). However, this test cannot differentiate among high and low *in vitro* and *in vivo* passages of the same *Lawsonia* isolate or between a vaccine isolate and its parent form (Weber *et al.*, 2004). The VNTR profile consisting of non-essential, intergenic DNA repeats within four loci remains stable over time even when manipulated through biological processes (Weber *et al.*, 2004). Therefore, the VNTR assay has been proposed to be an efficient research tool for tracking different genotypic subtypes of *L. intracellularis* among animal species and for possibly determining their epidemiological relatedness (Weber *et al.*, 2004).

Prevalence of disease

It has been determined that pigs on 20–50% of farms worldwide are infected with *L. intracellularis* (Stege *et al.*, 2000; Chouet *et al.*, 2003; McOrist *et al.*, 2003; Suto *et al.*, 2004). The disease may be more prevalent in the US (96%) and Northern Europe (70–90%) compared to Southern Europe (50–70%) where higher percentages of pigs are positive for PE based on serological prevalence data (McOrist *et al.*, 2003). A longitudinal study of a natural *L. intracellularis* infection in five large Danish pig herds revealed that the bacterium was present in all herds, and 75% of pigs examined by PCR were actively infected (Stege *et al.*, 2004). In a cross-sectional study of eight growing swine herds (weaning to 24 weeks of age) and three breeding herds in the Midwestern United States, *L. intracellularis* exposure was observed by IPMA in 75% of growing herds and 78% of replacement gilt herds (Marsteller *et al.*, 2003).

Prevalence data depicting the true incidence of *L. intracellularis*-specific disease on farms can be skewed high or low depending on sampling times and the diagnostic assay used (PCR, IFAT and IPMA). Previous field and controlled challenge exposure studies have shown that pigs were positive by PCR on feces 1–2 weeks before they were IPMA positive to *L. intracellularis* (Guedes *et al.*, 2002b; Guedes and Gebhart, 2003a). However, other studies have revealed seroconversion to *L. intracellularis* before shedding was evident via PCR (Knittel *et al.*, 1998; Kroll *et al.*, 2004a). Guedes (2004) reported that pigs are probably in the early stages of infection or have not had time to mount a detectable humoral immune response when they are PCR positive but serologically negative for *L. intracellularis*. Likewise, when pigs are PCR negative but positive for antibodies, this may indicate previous exposure or lack of sensitivity in the PCR assay to detect *L. intracellularis* (Guedes, 2004). These observations stress the need for evaluating *L. intracellularis* prevalence on farms using both PCR and serology when possible (Guedes, 2004).

White breed hybrid stock seem to develop PE more readily than Duroc-cross pigs (McOrist *et al.*, 2003). Limited contact between sows and their offspring (segregated early weaning systems) and the movement of pigs in large groups (all in/all out systems) have influenced the dynamics of PE on farms (Bronsvoort *et al.*, 2001). Disease transmission is reduced early in life with consequent susceptibility to PE if introduced to them at a later developmental stage (McOrist *et al.*, 2003). *Lawsonia*-specific infections typically reach high levels in grower pigs at about 8 weeks of age and remain active throughout the finishing and breeding stages (Chouet *et al.*, 2003).

Modes of transmission

Transmission of *L. intracellularis* from pig to pig is efficiently accomplished by the fecal–oral route through high levels of bacteria in pig feces. The environment of many pig farms contains a sustained level of *L. intracellularis*, which allows the reintroduction of infection to new groups of pigs at various ages (McOrist *et al.*, 2003). Previous reports revealed that pig to pig contact contributed greatly to the transmission of acute PHE among breeding stock and recently weaned or adult pigs (Rowland and Rowntree, 1972; Love *et al.*, 1977). A controlled challenge exposure study revealed sentinel pigs became infected when housed in contact with pigs inoculated with a relatively low dose of pure culture *L. intracellularis* (Jordan *et al.*, 2004), confirming that pig feces is the main source of new infections in susceptible swine (Guedes, 2004). Transmission of *L. intracellularis* from sows to offspring is influenced by the age and parity of the sow (Mauch and Bilkei, 2004). In this evaluation, sera from 99 healthy late pregnant gilts and 98 sows of parity 3–5 were screened by IFAT for anti-*Lawsonia* antibodies (Mauch and Bilkei, 2004). The offspring of gilts showed strong seropositivity to *L. intracellularis* from 5 to 26 weeks of age, whereas offspring from parity 3–5 sows showed lower IFA values which declined more rapidly (Mauch and Bilkei, 2004). These results indicated that older, seropositive sows either do not excrete sufficient organisms to induce detectable seroconversion or protect their offspring passively with maternal antibodies (Bronsvoort *et al.*, 2001; Barna and Bilkei, 2003). Therefore, recently infected gilts and low-parity sows are the primary source of *L. intracellularis* infection for their piglets (Mauch and Bilkei, 2004).

Partial protection against long-term infection and reinfection may be present in offspring of seropositive gilts (Mauch and Bilkei, 2004). However, the longer lasting seropositivity of the offspring of naïve gilts suggests reinfection with *L. intracellularis* (Mauch and Bilkei, 2004). Other possible mechanisms of *L. intracellularis* transmission include fomites (rubber boots, coveralls, etc.) and biological vectors such as mice, birds and insects, and should be considered for future evaluations (Guedes, 2004).

Persistence

Persistence of *L. intracellularis* can be classified into two categories: environmental and within the host animal. Environmental persistence is due to the ability of *L. intracellularis* to remain alive and stable for long periods of time under the various conditions found in the field. Persistence within the host animal occurs because of the ability of *L. intracellularis* to successfully colonize and multiply while evading the host's immune response to infection over a period of time. Environmental survival of *L. intracellularis* in pig confinements is a key factor in reinfection among swine herds. However, due to the difficulty of isolating *L. intracellularis* from feces or PE-infected intestines, viability studies to determine the resistance of this organism to various environmental and antimicrobial stresses are limited. In one study, Collins *et al.* (2000) investigated the rates of intestinal colonization of *L. intracellularis* in pigs after oral inoculation with feces from *Lawsonia*-positive pigs. The *Lawsonia*-infected feces had been stored at various temperatures (between 5 and 15°C) for up to 2 weeks and were found by PCR and histology to be infectious when given to naïve pigs.

A recent study revealed that pigs can shed *L. intracellularis* intermittently for a period of 12 weeks after experimental inoculation (Guedes and Gebhart, 2003c). These results demonstrated the capability of long-term colonization and survival of *L. intracellularis* in the host animal (Guedes and Gebhart, 2003c). Pigs that carry and frequently shed the pathogen without presentation of clinical symptoms for long periods of time are considered to be subclinically infected with PE (Jacobson *et al.*, 2003b). A study comparing the clinical, morphological and microbial findings in animals from good and poor performance herds found that clinically healthy pigs were often infected by *L. intracellularis* (PCR +) and had poor growth performance compared to pigs in uninfected herds (Jacobson *et al.*, 2003b).

Immune responses and immunity to *L. intracellularis*

The pathogenesis of infection with *L. intracellularis*, an obligate intracellular mucosal pathogen that is transmitted by the fecal–oral route, suggests that a protective immune response against *L. intracellularis* infections would involve aspects of humoral, mucosal and cell-mediated immunity. Many authors have identified specific host immune reactions to natural and experimental *L. intracellularis* exposure, while some have identified possible mechanisms of protective immunity against PE in pigs.

Humoral immunity

The presence of serum antibodies against an intracellular bacterium was first described in studies conducted in hamsters where convalescent serum collected from those with severe lesions reacted with the intracellular bacteria embedded within affected tissue sections (Jacoby, 1978). An immunoassay was developed to detect serum IgA and IgM antibodies against *L. intracellularis*, but was found to detect antibodies only in pigs with severe lesions (Lawson *et al.*, 1988). The anti-*Lawsonia* antibodies detected in growing pigs were predominantly IgM and were short lived (Lawson *et al.*, 1988). Holyoake *et al.* (1994) developed an ELISA that was able to detect a weak anti-*Lawsonia* IgG antibody response in experimentally

challenged pigs around 2–3 weeks post-inoculation. Results in younger pigs from other studies revealed passively acquired IgG antibody at 3 weeks of age and apparent seroconversion between 7 and 24 weeks of age (Holyoake *et al.*, 1994). An IFA test proved to be more sensitive and specific in detecting IgG levels in pig sera (Knittel *et al.*, 1998). In these experiments, the majority of pigs (90%) seroconverted by 3 weeks following an experimental challenge with a virulent pure culture *L. intracellularis* (Knittel *et al.*, 1998). The duration of antibody persistence was not determined in these controlled exposure studies since the study typically ended around 3 weeks post-challenge. In a comparison study, serological responses and duration of antibody detection were evaluated among pigs that received virulent and avirulent (vaccine) isolates of *L. intracellularis* (Guedes and Gebhart, 2003a). Pigs challenged with virulent *L. intracellularis* elicited a *Lawsonia*-specific serum IgG response that was detected 2 weeks after inoculation and remained detectable by IPMA up to 13 weeks post-challenge (Guedes and Gebhart, 2003a). Pigs challenged with an avirulent *L. intracellularis* elicited a delayed *Lawsonia*-specific IgG response that was not detected until 5 weeks post-challenge (Guedes and Gebhart, 2003a). A delay in a serum antibody response and lower peak IgG titers (3280 and 480 for virulent and avirulent *L. intracellularis*-exposed pigs, respectively) may have been the result of host responses to two different types of antigen (from virulent and avirulent organisms), differences in quantity of *L. intracellularis* in each challenge or due to reinfection because of the higher rates and duration of shedding in the virulent *L. intracellularis* challenge group (Guedes and Gebhart, 2003a).

Previous studies involving the evaluation of an avirulent live *L. intracellularis* vaccine in pigs have demonstrated that protective immunity against PE does not rely on an efficient and robust humoral immune response (Kroll *et al.*, 2004a). In these experiments, in which the IFAT was used as the detection assay, pigs did not have detectable anti-*Lawsonia* IgG serum antibodies up to 5 weeks post-vaccination but were significantly protected against a virulent *L. intracellularis* challenge exposure (Kroll *et al.*, 2004a). These results were consistent with previous assessments that anti-*Lawsonia* serum IgG levels are not expected to correlate to protection because *L. intracellularis* is an obligate intracellular organism that resides in the cytoplasm of enterocytes (Guedes and Gebhart, 2003a). Local mucosal IgA levels and cell-mediated immune responses are probably more involved with protection against infection; therefore oral or intranasally delivered modified-live vaccines against *L. intracellularis* are preferred over parentally administered vaccines (Guedes and Gebhart, 2003a).

Maternally derived IgG, IgA and IgM antibodies specific for *L. intracellularis* may be important against early infections in recently weaned piglets. Results from a controlled challenge study revealed that the presence of IFAT-detectable IgG antibodies in piglets up to 5–6 weeks of age conferred significant maternal protection against a virulent *L. intracellularis* challenge exposure (Kroll *et al.*, 2005a). High levels of IgA and IgG and, to a lesser extent, IgM were found in the colostrum of hyperimmunized sows, indicating passive transfer of *Lawsonia*-specific maternal antibodies to piglets during the first few days of life (Kroll *et al.*, 2005a). Seropositivity of gilts resulted in protective maternal immunity for up to 3 weeks when their piglets were challenged with virulent *L. intracellularis* (Bilkei, 1996). It is likely that piglets having maternal antibody protection shed *L. intracellularis* for a shorter period of time compared to piglets born to *Lawsonia*-naïve gilts (Gebhart and Guedes, 2001). However, effective length of protection provided by maternal immunity for pigs has not yet been established (Gebhart and Guedes, 2001).

A strong positive association between the *Lawsonia* seropositivity of grower-finisher pigs and the serological status of their dams was determined by Bronsvoort *et al.* (2001). A positive *Lawsonia* serological status of the sows was associated with lower anti-*Lawsonia* IgG antibodies in their offspring as a result of lack of exposure or passive immunity to the organism (Winkelman, 1996). In a controlled challenge exposure study, seropositivity in offspring of IFAT-negative gilts was highest 3 weeks (84%) post-challenge and declined gradually to only 10% at 24 weeks post-challenge (Barna and Bilkei, 2003). At the same time, the offspring of IFAT-positive gilts showed lower and faster-decaying seroprevalence in which only 32% were IFAT positive at 3 weeks post-challenge and no anti-*Lawsonia* antibodies were detected by 15 weeks post-challenge (Barna and Bilkei, 2003). These evaluations suggest that maternally derived, passive immunity may give partial protection against *L. intracellularis* infections to the offspring of seropositive gilts.

Passive immunization in pigs using chicken egg yolk antibodies has been evaluated as an alternative method for prevention of enteric disease (Winkelman *et al.*, 2004). Large quantities of *Lawsonia*-specific antibodies secreted into the yolk of eggs from chickens hyperimmunized with purified, whole cell *L. intracellularis* antigen can be subsequently harvested and fed to pigs. In this study, chicken anti-*Lawsonia* antibody titers were detected up to 1 : 1920 in hen sera at 4 weeks after the first immunization, while egg yolk antibody titers were higher at 1 : 1000 to 1 : 10,000 (Winkelman *et al.*, 2004). Pigs that received 2 kg of chicken egg yolk with anti-*Lawsonia* antibodies had significant ($P<0.05$) increases in ADG (25%) and average daily feed intake (27%) compared to pigs that received a placebo after virulent challenge with a mucosal homogenate containing *L. intracellularis* (Winkelman *et al.*, 2004). However, no statistical differences were noted among treatment groups regarding clinical

symptoms, fecal shedding (PCR), gross and microscopic (IHC) lesions (Winkelman *et al*., 2004).

Mucosal immunity

Intestinal sections from pigs affected with PIA and PHE revealed immense accumulations of IgA in the apical cytoplasm of proliferating enterocytes (Lawson *et al*., 1979). These accumulations of IgA were also evident in the Peyer's patches (Lawson *et al*., 1979) and in the cytoplasm of plasma cells underlying severe proliferative lesions (Holyoake, 1993). In other studies, IgA antibodies were detected in macrophages and cell debris of the crypt lumen in PHE cases (McOrist *et al*., 1992). However, in all of these reports, the detection of IgA may not have been a specific response to *L. intracellularis*.

A study was conducted to observe the progression of an *L. intracellularis* infection through the course of the disease and evaluate the production of *Lawsonia*-specific mucosal secretory IgA (Guedes and Gebhart, 2002c). Immunoglobulin A titers (1:4) in intestinal gavages of infected pigs were first detected by day 15 post-challenge by a modified IPMA test. Detectable titers were evident up to day 29 post-challenge with IgA titers ranging from 1:4 to 1:16 in the affected pig gut (Guedes and Gebhart, 2002c). Positive IHC staining for *L. intracellularis*, microscopic lesions and crypt hyperplasia were evident up to day 29 post-challenge, thus correlating to the production and subsequent detection of IgA in the affected intestines (Guedes and Gebhart, 2002c). Further studies are warranted to evaluate the level of protective immunity and the IgA response against an *L. intracellularis* infection in pigs.

Cell-mediated immunity

The inflammatory response in pigs to PE is minimal, which is indicative of a well developed lamina propria (Rowland and Lawson, 1974). In previous observations, accumulations of eosinophils were observed throughout the mucosa of pigs in the early stages of hemorrhage associated with PHE (Love and Love, 1979). Various immunocytological evaluations involving intestinal sections from pigs affected with PE indicated that the initial cell-mediated immune response included a mild infiltration of cytotoxic and suppressor T cells, macrophages and B lymphocytes carrying MHC Class II motifs (McOrist *et al*., 1992). Pigs 12–16 weeks of age affected with PHE revealed moderate infiltration of mononuclear lymphoid cells and polymorphonuclear leukocytes in the lamina propria and in the dome area of the Peyer's patches (McOrist *et al*., 1992). Only a mild infiltration of $CD8^+$ and $CD25^+$ T cells was found in the lamina propria during cases of PIA, a slightly greater infiltration of these cells along with lamina propria IgM-positive B cells was

found in hemorrhagic lesions of PHE-affected pigs (McOrist *et al*., 1992). However, MacIntyre *et al*. (2003) demonstrated in a controlled challenge exposure study an association between the presence of *L. intracellularis* and reduced T cell and B cell numbers in affected pigs. These authors noted an inverse correlation between the level of *L. intracellularis* infection and lymphocyte populations; T cells decreased as *L. intracellularis* infection increased, indicating an immunosuppressive mechanism of this bacterium during pathogenesis. Despite an apparent suppression of immune responses, antigen-dependent lymphocyte mitogenicity of peripheral blood mononuclear cells from *Lawsonia*-infected pigs compared to non-infected pigs has demonstrated specificity of lymphocyte responses to *L. intracellularis* (McOrist *et al*., 1992, 1993).

Other studies have provided further evidence of a cell-mediated response in pigs exposed to virulent and vaccine isolates of *L. intracellularis* (Guedes and Gebhart, 2003a). Peripheral blood mononuclear lymphocytes were harvested weekly from pigs on days 9–91 post-inoculation and levels of interferon gamma (IFN-γ) were determined by an ELISPOT assay (Guedes and Gebhart, 2003a). *Lawsonia*-induced, IFN-γ-secreting T cell responses became detectable at day 9 post-inoculation in pigs that received 1.76×10^8 *L. intracellularis* ml^{-1} of virulent challenge and at day 28 post-inoculation in pigs that received 5.3×10^5 *L. intracellularis* ml^{-1} of an avirulent vaccine challenge (Guedes and Gebhart, 2003a). Pigs that were inoculated with the vaccine isolate showed a delayed and lower cell-mediated immune response when compared to pigs that received the virulent *L. intracellularis* challenge. However, differences in cell-mediated response among treatment groups may be explained similarly to those mentioned above regarding the humoral response in this study (Guedes and Gebhart, 2003a). Regardless of these differences, both virulent and avirulent isolates of *L. intracellularis* induced detectable cell-mediated immunity that lasted for at least 13 weeks in some animals (Guedes and Gebhart, 2003a). Significant roles for IFN-γ have been shown for other intracellular pathogens and from previous studies using a mouse *L. intracellularis* challenge model (Smith *et al*., 2000). Results from these experiments revealed that IFN-γ receptor knock out mice were substantially more susceptible and had higher levels of *L. intracellularis* infection and lesion development compared to wild-type mice (Smith *et al*., 2000). IFN-γ, a Th1 cytokine, is involved in directing immune responses towards a cell-mediated response when host cells are insulted by intracellular pathogens (Smith *et al*., 2000). Natural infections with *L. intracellularis* may stimulate IFN-γ-secreting lymphocytes similar to those in experimental exposure studies and these may be involved in natural clearance of infection (Smith *et al*., 2000).

In an *L. intracellularis* challenge model comparison study, pigs were given intradermal injections of various

concentrations of *L. intracellularis* antigen (formalin-fixed whole cell (10^7–10^9 ml^{-1}), sonicated fractions (25–250 µg ml^{-1}) or outer membrane proteins (OMPS) (7.5–75 µg ml^{-1})), 20 days after virulent challenge exposure (Guedes and Gebhart, 2002c). Delayed-type hypersensitivity (DTH) reactions involving the detection of skin reactions (reddening and swelling) and IFN-γ were evaluated 24 and 48 h post-inoculation (Guedes and Gebhart, 2002c). This type of DTH (tuberculin) typically occurs within 48–72 h in a sensitized host and activates antigen-specific T cells to secrete cytokines that mediate the hypersensitivity reaction (Roitt *et al.*, 1998). Results showed that *Lawsonia*-challenged pigs from both treatment groups (pure culture and mucosal homogenate) showed a dose-dependent DTH reaction to the formalin-fixed, whole cell *L. intracellularis* preparations that was more evident 24 h after injection (Guedes and Gebhart, 2002c). Further studies are necessary for determining *Lawsonia*-specific induction of immunological memory and its duration and how it correlates to protective immunity in pigs.

Control and prevention measures

Several risk factors have been previously defined that predispose pigs to *L. intracellularis* infections and PE. Various control and prevention measures such as the use of antimicrobials that are effective against *Lawsonia* have been implemented in pig production systems around the world and have been successful in reducing or controlling *L. intracellularis* infections and PE. However, increasing global pressures to reduce or eliminate the routine use of antibiotics as growth promoters and prophylactic agents in food-producing animals have forced farmers, producers and veterinarians to rethink how to effectively control enteric diseases such as PE. Significant changes in pig management including all in/all out production, three-site production, segregated early weaning, medicated early weaning and establishment of high health herds were designed to reduce pathogen exposure in pigs. Despite these management practices and the common use of antimicrobials in pig production, PE continues to be a major economic problem in swine systems today. Changes in diets and the use of vaccines and disinfectants have shown promise as viable alternatives for reducing and eliminating *L. intracellularis* in pigs without the continuous use of antimicrobials.

Diet

Several feeding strategies have been investigated to determine their level of influence on *L. intracellularis* infections in pigs (Boesen *et al.*, 2004). In three experimental trials, 144 weaned pigs were fed five different diets all derived from a standard diet based on wheat and barley as the primary carbohydrate source and soybean as the primary protein source (Boesen *et al.*, 2004). Experimental diets among the five treatment groups consisted of standard diets with the following modifications: (1) fine ground and pelleted, (2) coarse ground and non-pelleted, (3) fermented liquid feed, (4) addition of 1.8% formic acid and (5) addition of 2.4% lactic acid. The effects of fermenting, acidifying and grinding the feed on *L. intracellularis* colonization and the development of PE after experimental challenge with a gut homogenate containing a Danish isolate of *L. intracellularis* were investigated (Boesen *et al.*, 2004). The mean duration of fecal shedding of *L. intracellularis* was significantly ($P<0.05$) lower in pigs consuming a fermented liquid standard diet compared to pigs fed a non-fermented standard diet (Boesen *et al.*, 2004). All treatment groups had lower average daily weight gains compared to the non-infected controls. Histopathological examinations were uneventful as only a few pigs had *Lawsonia* antigen in the surface epithelium and surrounding macrophages (Boesen *et al.*, 2004). These results, along with decreasing PCR positives at the time of necropsy (4–5 weeks post-challenge), suggest that pigs were recovering from *L. intracellularis* infections as the peak infection period for this organism is generally 3–4 weeks post-exposure (McOrist *et al.*, 1996a, Jensen *et al.*, 1997). The authors concluded that the fermented liquid diet delayed the excretion of *L. intracellularis* and that pigs fed a diet supplemented with 2.4% lactic acid showed signs of limited pathological lesions when examined for gross lesions at 4 weeks post-challenge (Boesen *et al.*, 2004).

Disinfectants

Since *L. intracellularis* is effectively transmitted from pig to pig in the feces, reducing cross-contamination between pig groups would help reduce levels of infection and incidence of PE. Because contaminated feces can be transported on dirty boots, clothing and equipment, simple biosecurity measures such as use of disinfectants should be implemented (Guedes, 2004). Previous *ex vivo* evaluations of various disinfectants and their effects on *L. intracellularis* found the bacteria to be highly susceptible to 3% cetrimide (quaternary ammonium), mildly susceptible to 1% providone–iodine and resistant to 1% potassium peroxymonosulfate or a 0.33% phenolic mixture (Collins *et al.*, 2000). Currently, no scientific studies have been published that effectively demonstrate the killing rates of these and various other disinfectants on *L. intracellularis* in the field under various production systems. Such evaluations are extremely difficult to conduct due to the difficulties of isolating obligate intracellular organisms. Future studies are warranted to identify disinfectants that effectively reduce or eliminate *L. intracellularis* under various housing conditions, pig

manure and fomites such as boots, gloves and clothing. Also, it would be helpful to know the frequency with which anti-*Lawsonia* disinfectants should be applied in typical production settings as well as the duration of time for effective microbial killing under various environmental conditions.

Antibiotics

The challenges of isolating and maintaining *L. intracellularis* cultures *in vitro* have made antimicrobial susceptibility testing an extremely difficult task. Nonetheless, *in vitro* evaluations of the minimum inhibitory concentration (MIC) of 20 antimicrobial agents and the minimum bactericidal concentration (MBC) of 10 of these agents indicate a broad range of antibiotic activity against *L. intracellularis* (McOrist and Gebhart, 1995; McOrist *et al.*, 1995b). Included in this list are the macrolides (i.e. erythromycin and tylosin), tetracyclines, pleuromulins (i.e. tiamulin), penicillins, and fluoroquinolones (McOrist and Gebhart, 1995; McOrist *et al.*, 1995b). Antibiotics that have no activity against *L. intracellularis* include the aminoglycosides and aminocyclitols (i.e. neomycin, gentamicin and apramycin) (McOrist and Gebhart, 1995; McOrist *et al.*, 1995b).

Advancements in *L. intracellularis* pure culture challenge models and diagnostic detection systems have enabled *in vivo* antibiotic sensitivity trials to evaluate numerous medication protocols for effective treatment and prevention of PE. Oral administration of tiamulin in weaned pigs at 50 ppm (water) from 2 to 21 days pre- and post-challenge or at 150 ppm (feed) from 7 to 21 days pre- and post-challenge was effective in preventing microscopic lesion development and reduced clinical symptoms after a virulent pure culture *L. intracellularis* challenge (McOrist *et al.*, 1996b). In another study, tiamulin administered at an in-feed rate of 35 or 50 g ton^{-1} significantly ($P<0.05$) prevented the development of gross lesions in the ileum, significantly ($P<0.05$) reduced the prevalence and severity of microscopic lesions and significantly ($P<0.05$) reduced fecal shedding of *L. intracellularis* compared to the non-medicated, control group (Schwartz *et al.*, 1999). This study also identified significant ($P<0.05$) reductions in seroconversion to *L. intracellularis* in medicated pigs even though these pigs were shedding the organism in their feces during the early infection period (Schwartz *et al.*, 1999).

Oral administration of tylosin phosphate at 100 or 40 ppm in feed 4 days pre-challenge up to 20 days post-challenge followed by 40 or 20 ppm for an additional 12 days prevented *Lawsonia*-specific microscopic lesions (McOrist *et al.*, 1997b). Tylosin given to weaned pigs at 100 ppm in feed 7 days after challenge with a virulent pure culture *L. intracellularis* prevented lesion development (McOrist *et al.*, 1997b). Marstellar *et al.* (2000) demonstrated effective treatment and control of PE through marked reductions in diarrhea, quicker resolution of gross lesions in the ileum and significantly ($P<0.05$) decreasing microscopic lesions when administering Tylan 200 by intramuscular injection.

Chlortetracycline (CTC) administered to 4-week-old pigs at 300 and 600 ppm from 4 days pre-challenge to 21 days post-challenge prevented the development of gross and microscopic lesions of PE (McOrist *et al.*, 1999). Weaned pigs challenged with an oral dose of PHE-affected gut homogenate and treated with 500 ppm of CTC for 10 days followed by 100 ppm for 10 days at 2 weeks post-inoculation when diarrhea was evident, had significantly ($P<0.05$) less microscopic lesions of PE compared to controls (Winkelman *et al.*, 1997). Treatment with 100 ppm of CTC in feed at 2 weeks post-inoculation with a high dose of a gut homogenate failed to reduce the frequency of microscopic lesion development compared to non-medicated control pigs (Winkelman *et al.*, 1997).

Studies conducted by Shultz *et al.* (1997) revealed that continuous in-feed medication with bacitracin methylene disalicylate and CTC for 14 days after a virulent *L. intracellularis* challenge prevented clinical symptoms of PE. CTC at 110, 220 and 440 ppm respectively in feed was effective against development of PE; however, bacitracin methylene disalicylate alone will not prevent *L. intracellularis* infections and was added at 33 ppm to reduce the levels of confounding bacterial pathogens.

Lincomycin administered in feed at 44 and 110 ppm for 21 consecutive days beginning after the onset of clinical symptoms was effective in reducing diarrhea while increasing ADG and feed conversion efficiency after a virulent *L. intracellularis* gut homogenate challenge (Winkelman *et al.*, 2002). Lincomycin was effective at significantly ($P<0.05$) reducing PE-specific mortality at 110 ppm only (Winkelman *et al.*, 2002). However, lincomycin at both concentrations was unable to prevent histological lesions of PE in this study. Lincomycin given to pigs at 200 ppm in feed from 7 to 21 days post-challenge with a virulent gut homogenate containing *L. intracellularis* effectively controlled clinical symptoms and reduced the severity of microscopic lesions (Winkelman *et al.*, 1998). However, these results indicated that overall microscopic lesion development was not significantly reduced in medicated pigs compared to non-medicated control pigs (Winkelman *et al.*, 1998). Medicated pigs showed reduced levels but not elimination of fecal shedding 2–4 weeks post-challenge (Winkelman *et al.*, 1998).

Increases in average daily weight gains and average daily feed intake were evident in 3–4-week-old pigs receiving 44, 88 or 132 ppm of lincospectin (lincomycin and spectinomycin) in the feed after a virulent pure culture challenge of *L. intracellularis* (McOrist *et al.*, 2000). In another study, treatment with 125 or 250 ppm of doxycycline for 14 days in 3–4-week-old weaned pigs had beneficial effects in terms of reductions in diarrhea, prevalence of *L. intracellularis* in intestinal tissue (PCR

or histological analysis) and increases in growth performance compared to non-medicated control pigs (Kyriakis *et al.*, 2002a).

Josamycine, belonging to the therapeutic class of macrolides, had beneficial effects against PE under field conditions when administered to weaned pigs 3–4 weeks of age (Kyriakis *et al.*, 2002b). Inclusion levels of 36 and 50 mg kg^{-1} of feed reduced diarrhea and prevalence of *L. intracellularis* in the intestine (PCR and histological analysis) while enhancing growth performance in medicated pigs compared to non-medicated, control pigs (Kyriakis *et al.*, 2002b).

Several studies have been conducted to evaluate the effectiveness of valnemulin hydrochloride (1.45–3.75 kg mg^{-1} of body weight for 7 to 21 consecutive days post-exposure) for the control of PE after a mucosal gut homogenate containing virulent *L. intracellularis* (Winkelman *et al.*, 2000a), when given simultaneously during an experimental *L. intracellularis* challenge (Winkelman *et al.*, 2000b) and during naturally occurring outbreaks of PE in Denmark (Haugegaard *et al.*, 2000). Results from these studies have shown that valenemulin hydrochloride was effective in the control of PE at 25–50 ppm in the feed by reducing clinical symptoms (diarrhea) and significantly (*P*<0.05) improving weight gain during the treatment period.

The most common treatment of PHE in adult pigs is tiamulin at 120 ppm, tylosin at 100 ppm, licomycin at 110 ppm, or CTC at 300 ppm for 14 days delivered orally in pre-mixed feed (McOrist and Gebhart, 1999). Where PE is endemic in grower-finisher pigs, the preferred treatment is continuous in-feed medication with tiamulin at 50 ppm, CTC at 200 ppm, lincomycin at 110 ppm and tylosin at 100 ppm to minimize severe production losses caused by the disease (McOrist and Gebhart, 1999).

Vaccines

Immunological control of intracellular pathogens commonly involves cell-mediated responses and is likely the primary factor in the control and prevention of *L. intracellularis* whether stimulation comes from inactivated, avirulent live, subunit or other vaccine types. Regardless of which approach is taken in the development, an effective vaccine must protect against multiple strains of the pathogen. Presently, *L. intracellularis* is considered a monotypic, single strain organism with no known antigenic variation among various isolates around the world (McOrist *et al.*, 2003).

Multiple genomic and proteomic evaluations over the years have confirmed that *L. intracellularis* is monotypic. Western blots of six antigenic OMPs of 77, 69, 54, 42, 36 and 18 kDa reacted similarly to monoclonal antibodies and convalescent serum from pigs previously exposed to virulent *L. intracellularis* (McOrist *et al.*, 1987; Guedes and Gebhart, 2003c). Additionally, these antigenic profiles

are conserved among six isolates of *L. intracellularis* of differing host sources and geographical origins (Guedes and Gebhart, 2003c). Monoclonal antibodies generated against *Lawsonia* surface antigens, LsaA and LPS, have been used as the primary detection antibodies in histological assays which have been successful in detecting various field isolates from affected tissues of pigs and other animals (Smith and Lawson, 2001; Boesen *et al.*, 2005a). Primer sequences based on the highly conserved 16 S rDNA gene of *L. intracellularis* used in the standard PCR protocol for detecting *Lawsonia* DNA in feces and tissue detected all isolates of US and European origins that were examined (Knittel *et al.*, 1996). Furthermore, these isolates shared the same morphological features and growth characteristics *in vitro* (Knittel *et al.*, 1996). Japanese researchers have reported that genetic sequences of three potential virulence factors or antigens (superoxide dismutase, LsaA and a 50-kDa OMP) derived from a Japanese porcine isolate of *L. intracellularis* show >99% homology to those of a UK isolate, NCTC 12657 (Koyama *et al.*, 2004). These results suggest high genetic similarity among various isolates of *L. intracellularis*.

The completion of the *L. intracellularis* genome sequence project will allow researchers to identify gene sequences involved in pathogenicity that could be viable targets for subunit vaccine approaches (Gebhart and Kapur, 2003). A partial DNA library was developed for obtaining clones for production of material for taxonomic, diagnostic and pathogenesis studies in addition to identifying potential protective antigens (Dale *et al.*, 1998). Thus far, no information has been reported defining potential immunogens as possible vaccine candidates.

Conventional avirulent live vaccines have been the only approach proven successful for developing protective immunity towards intracellular pathogens such as *Brucella* spp. and *Chlamydia* spp. (Su *et al.*, 2000; Morrison and Caldwell, 2002; Ko and Splitter, 2003). Killed or subunit vaccine prototypes against intracellular agents (*Chlamydia* and *Brucella*) have been futile (Shaw *et al.*, 2002; Ko and Splitter, 2003). Currently, an avirulent live *L. intracellularis* vaccine (Enterisol$^{®}$ Ileitis) was developed by Boehringer Ingelheim Vetmedica, Inc. for use in pigs 3 weeks of age or older for control and prevention of gross and microscopic lesions caused by *L. intracellularis* (Knittel and Roof, 1999; Kroll *et al.*, 2004a). This vaccine has been reported to stimulate both humoral and cell-mediated immunity in pigs; however, a direct correlation between the immune responses and the level of protection has not been established (Guedes and Gebhart, 2003a; Kroll *et al.*, 2005b). The *L. intracellularis* avirulent live vaccine was easily and effectively administered orally through the drinking water and resulted in significant (*P*<0.05) reductions in the prevalence and severity of gross and microscopic (IHC) lesions, colonization (PCR/IHC) and fecal shedding (PCR), while significantly

(*P*<0.05) improving average daily weight gains in pigs challenged with a virulent pure culture (Kroll *et al.*, 2004a). Onset and duration of immunity of this vaccine appears to be 3–4 weeks and ⩾22 weeks post-vaccination, respectively (Kroll *et al.*, 2004b). Various global field trials evaluating the efficacy of Enterisol® Ileitis have revealed significant (*P*<0.05) benefits in growth performance and reductions in % mortality against a natural *L. intracellularis* exposure in pigs (Sick *et al.*, 2002; Keita *et al.*, 2004; Kolb *et al.*, 2004). Safety of Enterisol® Ileitis has been described in pregnant sows (Kroll *et al.*, 2005a) and in studies consisting of inoculating pigs with repeat doses and an overdose, administration in 1-week-old piglets, and demonstrating a lack of reversion to virulence (Kroll *et al.*, 2004b).

Conclusions

PE, an enteric disease of pigs and several other animal species, includes many different syndromes, all of which are caused by a monotypic obligate intracellular organism, *L. intracellularis*. Hallmarks of the disease include severe thickening of the mucosal epithelia of the small and sometimes the large intestines. This lesion is commonly illustrated as a 'garden hose' because of the thick corrugated appearance of the affected tissue. Crypt hyperplasia and absence of goblet cells are due to unregulated proliferation caused by *L. intracellularis* infections.

The intracellular bacteria can be cultivated in adherent or suspension co-culturing eukaryotic cell systems under reduced oxygen or anaerobic conditions. Advancements in the growth and propagation of *L. intracellularis* within controlled suspension systems have allowed scientists to develop pure culture challenge models. These models provide a basis for better understanding of the pathogenesis of PE as well as the genotypic and phenotypic characteristics of the bacterium. Models also allow researchers to define processes for attenuated live vaccine development, and to develop new and improved diagnostic techniques.

Lawsonia-specific diagnostic methods have improved to levels where veterinarians and producers can now accurately and consistently determine the onset of exposure and prevalence of *L. intracellularis* in swine farms. Diagnosis of PE in pigs is no longer restricted to *post mortem* analysis. Molecular-based techniques like real-time PCR and serological tools like IFAT, IPMA and ELISAs have increased our knowledge of disease prevalence and transmission among swine farms while speeding up the process of receiving high quality results in a timely fashion.

PE is endemic among swine farms all over the world, having significant impact on growth performance and resulting in major financial losses for producers. Various antibiotics are commercially available that have

significant antimicrobial activity against *L. intracellularis* and aid in the control and prevention of PE. Recently, an avirulent live *L. intracellularis* vaccine was made available to the global swine market for use as an alternative tool or in conjunction with antibiotics through prescribed strategies for effective control and prevention of PE.

Despite all the significant advancements made in the understanding of *L. intracellularis* and PE, much remains to be resolved regarding pathogenesis, identification of metabolic and virulence characteristics, and immune responses due to wild-type and vaccine exposure in pigs. Future research should focus on bioinformatics and the utilization of the genome sequence for identifying and characterizing important immunogens, enhancing molecular techniques for epidemiological research and improving growth *in vitro*.

Acknowledgments

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