Detection and transmission of Lawsonia intracellularis in swine

Dianna Marie
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Recommended Citation
https://lib.dr.iastate.edu/rtd/17855

This Thesis is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Detection and transmission of *Lawsonia intracellularis* in swine

by

Dianna Marie Murphy Jordan

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Veterinary Microbiology
Major Professor: Lorraine J. Hoffman

Iowa State University
Ames, Iowa
1998
Graduate College
Iowa State University

This is to certify that the Master’s thesis of

Dianna Marie Murphy Jordan

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
DEDICATION

Daniel & Craig
Mom & Dad
Marilyn & Patrick
AND
ISU CVM Class of 1998
# Table of Contents

**General Introduction**

- Introduction ........................................................ 1
- Thesis Organization .............................................. 2

**Literature Review**

- Introduction .................................................. 3
- Economics and Impact ........................................ 3
- Incidence .......................................................... 4
- Etiology ............................................................ 4
- Terminology ....................................................... 4
- Disease and Clinical Manifestations ...................... 5
- Transmission ...................................................... 6
- Antimicrobials .................................................. 7
- Pathology .......................................................... 7
- Pathogenesis ....................................................... 8
- Diagnosis/Detection ............................................ 9
  - Histopathologic staining .................................. 9
  - Polymerase Chain Reaction ............................... 9
  - Serology ........................................................ 11
- Bibliography ..................................................... 11

**Detection of Lawsonia intracellularis in Swine Using Polymerase Chain Reaction Methodology**

- Abstract ......................................................... 18
- Introduction ..................................................... 19
- Materials & Methods .......................................... 21
  - Experimental Design ....................................... 21
  - Polymerase Chain Reaction ............................... 21
  - Histopathology ............................................... 22
# Table of Contents

Indirect Fluorescent Antibody Test (IFAT) .......................................................... 22  
Clinical Histories .................................................................................................. 22  
Results .................................................................................................................. 23  
Discussion ............................................................................................................ 24  
Acknowledgments ................................................................................................. 27  
References ............................................................................................................. 27  
Sources and Manufacturers .................................................................................... 35  

**A Lawsonia intracellularis Transmission Study Using a Pure Culture Inoculated Seeder-Pig Sentinel Model** ................................................................. 36  

**Summary** ........................................................................................................... 36  
- Objective .............................................................................................................. 36  
- Methods ............................................................................................................... 36  
- Results .................................................................................................................. 37  
- Implications ......................................................................................................... 37  

**Introduction** ...................................................................................................... 37  
**General Materials and Methods** ....................................................................... 38  

**Trial 1** ................................................................................................................ 40  
- Materials and Methods ....................................................................................... 40  
- Results .................................................................................................................. 40  
- Discussion .......................................................................................................... 41  

**Trial 2** ................................................................................................................ 42  
- Materials and Methods ....................................................................................... 42  
- Results .................................................................................................................. 42  
- Discussion .......................................................................................................... 43  

**Trial 3** ................................................................................................................ 43  
- Materials and Methods ....................................................................................... 43  
- Results .................................................................................................................. 44  
- Discussion .......................................................................................................... 44  

**Trial 4** ................................................................................................................ 44
Materials and Methods ................................................................. 44
Results .................................................................................. 45
Discussion ............................................................................. 45
Conclusions ............................................................................. 46
Implications ............................................................................. 48
Acknowledgments .................................................................. 48
References ............................................................................. 49

GENERAL CONCLUSIONS .......................................................... 62
ACKNOWLEDGMENTS .............................................................. 64
GENERAL INTRODUCTION

Introduction

Porcine proliferative enteropathy is an important enteric disease in the swine industry. The etiologic agent responsible for this disease is *Lawsonia intracellularis*. Economic losses which occur are primarily due to poor feed conversion and lack of uniformity among groups of pigs. Conventionally, a diagnosis is based upon the postmortem finding of thickened intestines, particularly in the ileum. Histopathologic stains such as Warthin-Starry are utilized to demonstrate the curved intracellular bacteria in the crypt enterocytes.

Polymerase chain reaction (PCR) is a molecular method for detecting *L. intracellularis* in intestinal mucosal scrapings or fecal material. A survey was done using ileal mucosa sections that were submitted to the Iowa State University Veterinary Diagnostic Laboratory. PCR was performed on 621 ileal mucosa samples. From these samples, 26 were positive by PCR for the presence of *L. intracellularis*. A comparison was made with the conventional Warthin-Starry stain. The PCR methodology detected more positives than the stain, and it was also more specific. PCR can also be utilized in situations where the lesions are subtle or the tissues are not adequate for histopathology. Additionally, PCR can be used as an antemortem tool to detect *L. intracellularis* in feces.

Few studies have been conducted on the transmission and progression of the disease. Four transmission trials were completed using a pure culture inoculum of *L. intracellularis* in conventional pigs to study the pattern of fecal shedding of the organism, the transmissibility of the shedding, and the seroconversion that occurs during infection. From these trials, it was demonstrated that the shedding of the organism as detected by PCR is cyclical in nature, as is the accompanying diarrhea. These cycles follow one another, but do not occur simultaneously. Successful infection does not always result in clinical signs or gross lesions, but low colonization in the intestine may lead to transmission of the organism and/or seroconversion.

These studies reaffirm that *L. intracellularis* is a challenging pathogenic bacterium to investigate. PCR has been demonstrated as a valuable diagnostic tool for detection of the
*L. intracellularis* in feces and mucosa samples. PCR results combined with serologic information are valuable for swine producers.

**Thesis Organization**

The thesis contains a general introduction, a literature review of porcine proliferative enteropathy due to *Lawsonia intracellularis*, two manuscripts submitted for publication, general conclusions, and acknowledgments. The first manuscript has been accepted for publication in the Journal of Veterinary Diagnostic Investigations and was supported by a Merck Scholarship Grant. Additional support came from Boehringer Ingelheim / NOBL Laboratories, Inc. and Iowa State University. The second manuscript will be submitted for publication to Swine Health and Production. This study fulfilled a research grant awarded from the National Pork Producers Council. Additional support was provided by the Iowa Livestock Health Advisory Committee and Boehringer Ingelheim / NOBL Laboratories, Inc.
Introduction

Porcine proliferative enteropathy (PPE) is a major enteric disease in swine. Although some deaths are associated with this syndrome, the primary financial losses are thought to be due to poor feed conversion, impaired rate of gain and lack of uniformity in large pig groups. The causative agent of PPE is *Lawsonia intracellularis*, a gram negative, obligate intracellular bacterium. Traditionally, diagnosis is based on postmortem examination and histopathology. More recently techniques such as polymerase chain reaction (PCR) and immunofluorescent antibody test (IFAT) serologic assays have been utilized for antemortem diagnosis and epidemiological surveillance. It is hoped that these tools will assist in gathering knowledge about the pathogenesis and transmission of the organism as well as provide insight for improved protocols of treatment and prevention of ileitis.

Economics and Impact

The disease which results from *L. intracellularis* infection can affect pigs at any stage of production, however, the major impact occurs during the late nursery and grower-finisher stages. The economic losses incurred from PPE include pig mortality, impaired feed conversion, decreased rate of gain, and medication costs. Stunting of affected pigs, a common manifestation of this syndrome, makes all-in-all-out production schemes less achievable and often results in high cull rates or marketing of underweight pigs. Reductions in feed conversion have been estimated between 20 and 30 percent and weight reductions up to 50% compared to normal pigs (Gogolewski et al 1991). The prevalence and subsequent financial impact of the disease has not been well documented, but the economic losses are estimated to be as much as $10 to $20 million annually (Mapother et al 1987). The inability to accurately assess the dollars spent on this syndrome is partially due to the lack of a valid antemortem diagnostic test (Jones et al 1993b; Rowland and Lawson 1992).
Incidence

Previous studies have shown a low prevalence of PPE in slaughterhouse pigs (0.7% to 1.63%) (Emsbo 1951; Kubo et al 1984; Rowland 1978). Reports published in the 1980’s suggest that 5% to 20% of pigs had lesions at slaughter with the prevalence reaching 40% in some herds (Pointon 1989). In 1991 it was estimated that 20 - 30% of herds may have the disease in a given year with 5-20% of the pigs affected (McOrist and Lawson 1990). In a PCR study of ilea submitted to the Iowa State University Veterinary Diagnostic Laboratory in 1995, 4% of samples taken from a wide age range of swine were positive (Jordan et al 1998).

Etiology

*Lawsonia intracellularis* is a microaerophilic, gram negative, curved bacterium. It is an obligate intracellular organism that requires cell culture and reduced oxygen tension for growth (Lawson et al 1993). This organism can be cultivated in intestinal epithelial tissue culture cells, but it produces no cytopathic effect on tissue monolayers (Knittel et al 1996). The laborious cell culture techniques required are not conducive for routine diagnosis.

*Lawsonia intracellularis* has been found in a number of species, including pigs, hamsters, horses, deer (Drolet et al 1996; Rowland and Lawson 1992), ostriches, and emus (Cooper et al 1997). These potential reservoirs could impact management of free ranging animals as well as interspecies rearing (Cooper et al 1997).

Terminology

Although PPE was described several decades ago, the agent responsible was recently named. Early in the study of the disease, the etiology was thought to be a *Campylobacter* species. In 1982, it was proposed that the organism should be referred to as *Campylobacter sputorum* subspecies *mucosalis* or CSM-like organism (Lomax and Glock 1982). In 1989 it was found that *Campylobacter mucosalis, C. hyointestinalis, C.jejuni,* or *C. coli* did not elicit clinical signs typical of the disease; thus, *Campylobacter*-like organism (CLO) was the acceptable designation (McOrist et al 1989b). That same year, it was shown serologically that
the CLO differed significantly from *Campylobacter* species. By dosing pigs with pure *Campylobacter* inocula which resulted in no disease and no corresponding anti-*Campylobacter* antibody reaction with the intercellular bacteria while there was a reaction with the anti-CLO antibody (McOrist et al 1989a). This suggested a novel bacterium. In 1990, restriction enzyme analysis and DNA-DNA blot hybridization techniques demonstrated fundamental differences in the fragment patterns of the CLO and *Campylobacter*. *Campylobacter intracellularare* was the suggested name at this point (McOrist et al 1990). In 1993, it was proposed that the CLO be given a vernacular name of Ileal Symbiont intracellularis. By using 16S rRNA gene sequencing and DNA probes, IS-intracellularis was found to have 91% similarity to a sulfate reducing proteobacterium *Desulfovibrio desulfuricans* and little similarity to *Campylobacter* (Gebhart et al 1993a). In 1995, the bacterium was officially named *Lawsonia intracellularis* after Gordon Lawson, who conducted much of the early work on the organism (McOrist et al 1995a). Koch’s postulates have been achieved using a pure culture of this agent; it is pathogenic for pigs and causes proliferative enteritis in the ileum and sometimes in the jejunum and colon (McOrist et al 1995a).

**Disease and Clinical Manifestations**

Synonyms of PPE used over the years include: ileitis, garden hose gut, proliferative enteritis, regional enteritis, and intestinal adenomatosis (Moore and Shryock 1996). PPE is a transmissible enteric disease of swine (Gebhart et al 1993b). It generally occurs in pigs between 6 and 20 weeks of age (McOrist et al 1994a; Rowland and Lawson 1992) and was first recognized in 1931 (Biester and Schwarte 1931). Several risk factors are identified for grower-finisher pigs including PPE naive stock, temperature fluctuations, relocation, commingling, and poor sanitation. Many of these factors are common in pig production facilities (Winkleman 1996). It is speculated that a higher prevalence of disease in the first six months of repopulation is common because little to no maternal or active immunity exists. All-in-all-out production decreases exposure to adult populations which may be harboring the organism, thus creating naive populations (Connor 1991).
The clinical signs of PPE are variable. It is not uncommon to observe acute death, diarrhea, or chronic weight loss in affected animals. Infected animals may not show clinical signs and those with mild clinical disease often recover without treatment intervention (Gebhart et al 1993b).

There are three recognized pathologic forms of PPE: porcine intestinal adenomatosis (PIA), porcine hemorrhagic enteropathy (PHE), and necrotic enteritis (NE) (Rowland and Lawson 1992). Clinically, there are three forms as well: acute, chronic, and inapparent (Ward and Winkelman 1990a). The most common sign of disease associated with the acute form is sudden death with or without reddish, black, or tarry feces (Rudolphi 1995; Ward and Winkelman 1990b). Usually this form will have the accompanying PHE lesions. The chronic form manifests itself as loose brown-gray watery diarrhea without mucous, but may have blood; the animals appear otherwise healthy but fail to gain weight properly (Ward and Winkelman 1990b). Other animals that are inapparently infected are gaunt, anemic, and listless (Ward and Winkelman 1990b). The major producer concern is lack of uniformity and feed efficiency within pig groups (Moore and Shryock 1996).

**Transmission**

Porcine proliferative enteropathy can be reproduced by feeding pigs intestinal homogenates from typical lesions. Intestinal homogenates were used in many investigations until pure culture propagation was successful. Lack of consistency and mixed populations of bacteria are major disadvantages of homogenate preparations. Tissue culture systems have been developed for the cultivation of the bacteria in high quantities. Clinical disease and lesions are reproducible by feeding pure cultures of *L. intracellularis* to conventional pigs (McOrist et al 1993), however, PPE cannot be reproduced in germ-free pigs with a pure culture inoculum. Normal flora, especially *Bacteroides vulgatus* and *Escherichia coli*, is required for infection to be established (McOrist et al 1994a). The disease is also transmissible from an intragastrically inoculated pig to a naïve sentinel pig when a live, pure culture of *L.intracellularis* is used (Jordan et al 1998). Fecal shedding of the bacteria and colonization are not directly related to the severity of diarrhea and other clinical signs. Pigs
that are colonized do not consistently shed detectable numbers of bacteria or display signs of diarrhea. Also, infected diarrheic pigs don't always shed organisms. Thus the diagnostic tests such as lesion detection (gross or histopathologic), PCR, and serology cannot be correlated with the degree of infection or shedding potential (Jordan et al 1998; Knittel et al 1997).

**Antimicrobials**

Antimicrobial agents capable of entering the host cells are the most likely candidates to be effective against *L. intracellularis*. McOrist has shown that several classes of agents including macrolides, tetracyclines, fluoroquinolones, tiamulin (a pleuromulin), and virginiamycin have activity against *L. intracellularis in vitro* (McOrist and Gebhart 1995). Minimal inhibitory concentration (MIC) values were obtained for various antimicrobials against *L. intracellularis*. The most active compounds, with low to moderate MIC values were erythromycin, difloxacin, virginiamycin, and chlortetracycline. The penicillins and fluoroquinolones were found to have low MIC and MBC (minimal bactericidal concentration) values but have not been widely recommended to treat PPE. An intermediate susceptibility response was obtained with tiamulin and tilmicosin (McOrist and Gebhart 1995). Aminoglycosides, lincomycin, and tylosin were relatively inactive as determined by in vitro MIC testing (McOrist et al 1995b). The MIC and MBC values were thought to reflect the true susceptibility of *L. intracellularis* to the drugs tested (McOrist and Gebhart 1995).

**Pathology**

Although variable in severity, the lesions of proliferative enteritis are characteristic. This pathologic consistency often allows for a confident diagnosis upon necropsy (Ward and Winkelman 1990b). Grossly the mucosa is thickened with corrugated folds and may be covered with a fibrinous membrane (Ward and Winkelman 1990b). The walls of the affected intestines are thickened (Gebhart et al 1993b). The affected intestines have an increased diameter and are rigid with a corrugated serosal surface with subserosal edema (Ward and Winkelman 1990b). There is a significant association of macroscopically thickened intestines and microscopic proliferative changes. The presence of edema in the intestines and the
mesentery only increased the significance of this association (Jones et al 1993a). In all manifestations, intestinal cryptal epithelial cells proliferate and/or fail to mature so that the mucosal crypts are elongated, enlarged, and are lined by immature epithelial cells (Gebhart et al 1993b). There are hyperplastic crypt enterocytes with numerous mitotic figures and an absence or decreased number of goblet cells. In the apical cytoplasm of the proliferating enterocytes, small, curved, rod-shaped intracellular bacteria can be observed with appropriate staining (Gebhart et al 1993b; Jones et al 1993b; McOrist et al 1994b; McOrist et al 1990; Rowland and Lawson 1992; Ward and Winkelman 1990b).

Pathogenesis

The transmission of *L. intracellularis* occurs via oral exposure of animals to infected mucosa or feces (Gebhart et al 1993a). The bacteria attach to crypt epithelial cells at the brush border. The organisms gain entry to the cells via vacuoles formed at the brush border. Once inside the cell, the vacuoles are rapidly broken down or they may coalesce with other vacuoles which eventually lyse. When the vacuoles are compromised, the bacteria are released into the cytoplasm. In the cytoplasm, multiplication occurs. Electron microscopy demonstrates the multiplying bacteria closely associated with mitochondria or rough endoplasmic reticulum which appear disrupted or markedly distended. This close association is thought to facilitate nutrient transfer (Jasni et al 1994). The bacteria seem to gain entry to the cells at five days post infection; hyperplasia is visible at ten days post-infection. As with many obligate intracellular parasites, bacterial release and host cell reinfection is common (Johnson and Jacoby 1978).

Hyperplastic lesions develop two to three weeks post-challenge and may persist for weeks (McOrist et al 1996a). The pathogenesis has not yet been determined, although there have been some hypothesis developed. McOrist serially examined pigs at different stages of the disease and evaluated the ultrastructural details of colonization, lesion development and resolution (McOrist et al 1996a). Possible pathogenic mechanisms that have been proposed by McOrist include: (1) local bacterial regulation of genes active in cell differentiation or apoptosis, (2) bacterial production of a mitogenic agent, (3) bacterial damage to cells creating
a wound healing proliferative response, and (4) bacterial alteration of a receptor-signaling mechanism related to normal growth factors (McOrist et al 1996a).

**Diagnosis/Detection**

There are other entities that can produce an inflammatory intestinal lesion or crypt cell hyperplasia; therefore, an inaccurate diagnosis could result if it is based on a lesion of rather than the presence of an organism. Identification of *L. intracellularis* is critical for the confirmation of PPE. Diagnostic detection methods include histopathology, PCR, fluorescent antibody, as well as serology for detection of antibodies against the organism.

**Histopathologic staining**

The inability of *L. intracellularis* to grow on artificial media presents a challenge for diagnosticians when attempting to confirm its presence; therefore, pathologic lesions and the accompanying presence of the organism in sections of fixed tissue are the conventional criteria used in the diagnosis of *L. intracellularis*.

The Warthin-Starry stain is used to demonstrate bacteria in formalin fixed intestines. With this silver impregnation technique, *L. intracellularis* appears as a dark, curved organism in the apical cytoplasm of the enterocytes. However, this stain is not specific for *L. intracellularis*.

The indirect fluorescent antibody test (IFAT) is specific for *L. intracellularis* and reveals organisms which may be not detected by the Warthin-Starry stain; although, there is still the histopathologic limitation of a small amount of tissue being examined.

**Polymerase Chain Reaction**

Polymerase chain reaction (PCR), a method which detects a specific and unique sequence of genetic material, is often useful in detecting organisms which are difficult or impossible to grow on conventional media (Jones et al 1993b). This rapid and specific test could be quite desirable for the detection of *L. intracellularis* in fecal and mucosal samples (Gebhart et al 1993b; Jones et al 1993b).
At this time, PCR on mucosal scrapings appears more sensitive and specific for *L. intracellularis* than the Warthin-Starry stain. There are several factors which may account for this apparent difference in sensitivity of PCR. The molecular technique may be detecting the organism prior to lesion development or after bacterial cell integrity is lost. Additionally, the PCR preparation represents an increased sampling area of the intestine, which potentially increases the cell mass of bacteria whereas a histopathologic section represents only a 5 micron cross-section of intestine which may not contain bacteria. PCR is capable of detecting $10^1$ to $10^4$ organisms per gram of sample on an ethidium bromide stained gel, detectable by a 319-bp band (Jones et al 1993b).

Primers for the specific unique sequences of DNA that detects *L. intracellularis* were developed by Gebhart, Jones, Cooper and coworkers (Gebhart et al 1991; Gebhart et al 1993a; Jones et al 1993b). There is a set of internal 16S ribosomal primers and a set of external chromosomal primers. The external primers were developed from a cloned genomic 375-bp segment from a *L. intracellularis*-specific clone which has been shown to be sensitive and specific for the detection of *L. intracellularis* in the pig (Gebhart et al 1991; Jones et al 1993b). The internal primers were developed from the highly conserved 16S rDNA sequence of *L. intracellularis* (Gebhart et al 1993a). The standard protocol for amplification of a sample utilizes only the external primers; for enhanced sensitivity, reamplification using the internal primers as a nested set can be done (Jones et al 1993b).

PCR testing of fecal and mucosal material is a rapid and sensitive test for detecting *L. intracellularis* in swine (Gebhart et al 1993b; Jones et al 1993b). A study by McOrist et al validated the use of PCR for the detection of the intracellular bacteria associated with proliferative enteritis. Positive fecal results were only observed in those animals with active lesions of the disease (McOrist et al 1994b). There have been studies in which PCR tests have detected *L. intracellularis* without corresponding gross lesions (Jordan et al 1998; Knittel et al 1997). PCR can be used as an ante-mortem diagnostic tool to identify those pigs that are actively shedding the organism, but cannot detect the colonized pigs that are not shedding. False negative results by PCR on feces can occur due to the nature of cyclical shedding of the organism (Jordan et al 1998; Knittel et al 1997).
Although results of PCR on mucosal samples are generally correlated with histopathologic results, the validity of a positive or negative fecal PCR sample remains an unknown quantity. It is not known if transmissible levels of the organism are evading PCR fecal detection in a normal animal environment. Jones et al have reported a sensitivity of PCR to $10^1$ to $10^3$ organisms per gram (Jones et al 1993b). There has not been an extensive study of PCR to determine its validity and applicability as an antemortem fecal test.

**Serology**

Until recently, serological techniques have not proven effective (Holyoake et al 1994; Lawson et al 1988). The lack of usefulness was attributed to a weak immune response to the bacterial infection. Specific IgA and IgM were only detected in the serum of animals with advanced intestinal lesions (Lawson et al 1993; McOrist et al 1994b). Holyoake developed an ELISA utilizing percoll-gradient purified intestinal homogenate for *L. intracellularis* as the antigen to detect IgG titers. The results from this assay were somewhat inconclusive due to low numbers of pigs involved in the study as well as lack of purity of the antigen. Cross-reactivity could not be determined (Holyoake et al 1994). Knittel has developed a serologic assay that is an immunofluorescent antibody test to measure IgG titers (Knittel et al 1998). Both investigators noticed a pattern of antibody detection that wanes after three weeks of age with a rise in titer after six weeks of age. This observation suggests there is a source of protective maternal antibody in the early weeks followed by a window of opportunity for natural exposure into the grower period with an active immune response following. The serology test will be a useful tool when investigating the course of PPE.

**Bibliography**


DETECTION OF LAWSONIA INTRACELLULARIS IN SWINE USING POLYMERASE CHAIN REACTION METHODOLOGY

A paper received for Publication November 1, 1996 in Journal of Veterinary Diagnostic Investigation

Dianna M. Jordan, Jeffrey P. Knittle, Michael B. Roof, Kent Schwartz, David Larson, Lorraine J. Hoffman

Abstract

The polymerase chain reaction (PCR) was evaluated for its usefulness as a diagnostic tool to detect *Lawsonia intracellularis*. Porcine ilea were collected from swine cases submitted to the Iowa State University Veterinary Diagnostic Laboratory between December 1, 1994, and June 30, 1995. Sampling was random with no regard to health status as a selection criterion. There were 621 ileum scrapings evaluated using the PCR technique. Thirty-five of the samples were positive, either by PCR or conventional diagnostic methods, such as histology and Warthin-Starry silver stain. These 35 samples were further evaluated by indirect immunofluorescent antibody test (IFAT) to confirm the presence of *L. intracellularis* in the tissue sections. Of the 26 samples positive by PCR, 22 were positive by IFAT. Sixteen of the 22 were also positive when stained with Warthin-Starry and evaluated microscopically for typical bacteria. Nine of the original samples which were interpreted to be positive on diagnostic histologic exam using a standard H&E stain, but were negative by PCR, IFAT, and Warthin-Starry. PCR appears more sensitive and specific for *L. intracellularis* than Warthin-Starry stain and IFAT. This study provides evidence that PCR may have merit for use as a reference standard for the detection of *L. intracellularis*. PCR may be an appropriate monitoring tool for swine herds because it is a rapid procedure which

\* Boehringer Ingelheim / NOBL Laboratories, Inc.; Project Leader
\* Boehringer Ingelheim / NOBL Laboratories, Inc.; Director of Research and Development
\* Iowa State University; Veterinary Diagnostican
\* Iowa State University; Associate Professor, Veterinary Diagnostics Production Animal Medicine
\* Iowa State University; Professor, Veterinary Diagnostics Production Animal Medicine
could be applied to batch testing. Although the test is currently too laborious and expensive for routine diagnostic use, there may be situations in which it is justified due to the advantages of greater sensitivity and specificity that is inherent in PCR.

Introduction

Porcine proliferative enteropathy (PPE) is a transmissible enteric disease of swine. It generally occurs in pigs between 6-20 weeks of age and was first recognized in 1931.8,10 The clinical signs of PPE are variable. It is common to observe acute death, diarrhea, or chronic weight loss in affected animals, however, infected animals do not always show clinical signs. Pigs with mild clinical signs sometimes recover without treatment.

Upon necropsy, the gross lesions of PPE are often characteristic, but not pathognomonic. Typically, gross lesions associated with PPE include dilated and turgid intestines with corrugated serosal folding. Hemorrhage may or may not be present. The intestinal mucosa is hypertrophied with deep transverse folds.11 Histologically, mucosal intestinal crypts are elongated, enlarged and lined by hyperplastic epithelial cells. Epithelial cell proliferation results in mucosal thickening of affected portions of the ileum, jejunum and large intestine. There is hyperplasia of crypt enterocytes with numerous mitotic figures and an absence or decreased number of goblet cells. In the apical cytoplasm of the hyperplastic enterocytes, small, curved, rod-shaped intracellular bacteria can be observed with appropriate staining, i.e. Warthin-Starry silver stain.2,10,11 Studies have shown a significant statistical correlation between lesions of PPE and presence of Lawsonia intracellularis.4

The disease that results from L. intracellularis infection can affect pigs at any stage of production. However, the major impact occurs during the grower and finisher stages. The economic losses from PPE include pig mortality, impaired feed conversion, decreased rate of gain and medication costs. The variable weight gain, a common manifestation of this syndrome, makes all-in-all-out production schemes less achievable for producers of all sizes and often results in increased culling of animals from large production units. The prevalence and financial impact of the disease has not been well documented. This is partially due to the
lack of a valid antemortem diagnostic test to detect the causative bacterium and confirm a diagnosis.\textsuperscript{5,10}

*Lawsonia intracellularis* is an obligate intracellular bacterium that requires tissue culture for growth.\textsuperscript{6} The inability of this microorganism to grow on artificial media presents a challenge for diagnosticians when attempting to confirm its presence. Gross pathologic lesions and observation of the organism in sections of Warthin-Starry stained fixed tissue are the conventional criteria used in the diagnosis of *L. intracellularis*. The primary detection method for *L. intracellularis* in tissue sections is the Warthin-Starry silver stain. With this technique, *L. intracellularis* appears as a dark, curved organism in the apical cytoplasm of the enterocytes. This stain however, does not confirm the identity of *L. intracellularis* or differentiate it from *Campylobacter* species or other curved rods.

The polymerase chain reaction (PCR) is used to identify a specific genetic sequence. PCR is particularly useful for the detection of organisms which are difficult or impossible to grow on conventional media.\textsuperscript{5} Testing of fecal and mucosal material by PCR is a rapid and sensitive method for detecting *L. intracellularis* in swine.\textsuperscript{2,5} A previous study validated the use of PCR for the detection of *L. intracellularis* associated with proliferative enteropathy.\textsuperscript{9} Instead of relying on necropsy lesions and nonspecific stains, PCR may allow rapid, accurate detection of *L. intracellularis* in antemortem samples. The PCR test could facilitate monitoring for *L. intracellularis* in swine populations and help evaluate the efficacy of treatment and management systems.

The goal of this study was to evaluate PCR as a diagnostic technique to detect *Lawsonia intracellularis* in intestinal specimens submitted to the Iowa State University Veterinary Diagnostic Laboratory. Additionally, this information provides a rough estimate for the age distribution of pigs positive for *L. intracellularis*. 
Materials & Methods

Experimental Design

Porcine ileal scrapings (621) were collected at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) between December 1, 1994, and June 30, 1995 and were evaluated for *L. intracellularis* using the polymerase chain reaction assay. The sample selection was all inclusive and not limited to pigs with gross lesions of proliferative enteritis. The ilea were obtained from pigs of all ages with a multiplicity of histories, clinical signs, and lesions. Ileal mucosal scrapings were obtained and frozen at -70°C until they could be evaluated by PCR. Portions of each ileum were fixed in 10% neutral buffered formalin to be sectioned for histopathological examination.

Polymerase Chain Reaction

The method for DNA extraction from the mucosa samples was a commercial guanidine thiocyanate nucleic acid extraction kit. The extracted DNA was subjected to PCR using commercially available reagents and following the protocol previously described. The primer set used, 5'-TATGGCTGTCAAACTCCG-3' and 5'-TGAAGGTATTTGCTTCTCC-3', for the organism were the same as previously described. Cycle parameters for the first thermo-cycle were 93°C for 5 minutes, 55°C for 45 seconds, and 72°C for 45 seconds. Thirty-three additional cycles were performed as follows: 93°C for 45 seconds, 55°C for 45 seconds, and 72°C for 45 seconds. The final cycle was conducted at 93°C for 45 seconds, 55°C for 45 seconds, and 72°C for two minutes. The PCR products were evaluated by electrophoresis on a 4% agarose gel. The DNA was stained by ethidium bromide and visualized under ultraviolet light. Positive *L. intracellularis* samples yielded a 319-bp band. Amplification of the primary PCR product, the result of one thirty-five cycle amplification process, was performed on three samples.

---

This was done because the IFAT result was clearly positive, but the result of the initial PCR amplification was negative.

**Histopathology**

Formalin fixed ileum sections from PCR positive samples were evaluated histologically at the ISU VDL. Four slides were made of each section. One slide was stained with hematoxylin and eosin (H & E) and the second slide was stained with Warthin-Starry. The H & E slides were evaluated for lesions and the Warthin-Starry slides were designated as either positive or negative for the presence of curved rods in the apical cytoplasm of crypt enterocytes. The third and fourth slides were used for IFAT and retained for future evaluation, respectively. Samples that were diagnosed as PPE at the ISU VDL but negative by PCR were later re-evaluated microscopically.

**Indirect Fluorescent Antibody Test (IFAT)**

An indirect immunofluorescent antibody test with mouse-derived monoclonal IgG specific for *L. intracellularis* was used as described previously. The slides were evaluated with a fluorescent microscope for the presence of curved intracellular organisms in the crypt enterocytes. The slides were scored either positive or negative on the basis of fluorescing intracellular bacteria.

**Clinical Histories**

Histories were obtained on all PCR positive samples. A search for PPE diagnosed cases based on gross and microscopic examination was done on all ISU VDL cases to avoid missing false negative PCR samples. Ages, weights, and other pathologic findings were recorded. The ages and weights were classified into five categories: neonate, nursery, grower-finisher, mature, and no information given. These categories are summarized in Table 1.
Results

Twenty-six of the 621 ileal scrapings were positive for *L. intracellularis* by PCR (Table 2). Three of these samples were positive only on secondary amplification of the primary PCR product. Secondary amplification was done on these three samples due to strong positive IFAT results. The PCR product is a 319 bp band on the agarose gel. The positive PCR products are illustrated in Figure 1. Warthin-Starry stains revealed organisms characteristic of *L. intracellularis* on 16 of the 26 PCR positive samples using histological sections. The IFAT revealed organisms stained with the monoclonal antibody specific for *L. intracellularis* on those 16 as well as on six additional sections in the group of 26. The remaining four samples were positive by PCR and negative by both Warthin-Starry and fluorescent antibody stains. Demonstration of the organism was necessary for a histopathologic result to be interpreted as a PPE positive sample. Only those samples with an organism confirmation was considered, therefore the H&E results were not tabulated. Although proliferative lesions seen on H&E may be characteristic, they are not pathognomonic; therefore, a proliferative lesion without the organism present does not constitute a diagnosis of *L. intracellularis*.

The remaining 595 samples were negative by PCR; if the history or ISU VDL diagnosis was suggestive of PPE, histology slides were reexamined. Nine ilea in addition to the 26 identified above had demonstrated proliferative microscopic lesions and were diagnosed as PPE based on these lesions. However, because no organisms were found by Warthin-Starry or fluorescent antibody stains, these samples were considered to be negative for *L. intracellularis*.

There were no other consistent pathologic or bacteriologic findings among these 26 cases. Postmortem diagnoses ranged from pneumonia to PPE. The age distribution was also varied (Table 3). One positive sample was detected by PCR and IFAT in the neonate age category. This sample was from a two week old pig.
Discussion

There is no microbiological standard established for definitive identification of *L. intracellularis*. Diagnosis is made by Warthin-Starry silver impregnation stains to demonstrate curved intracellular organisms accompanying proliferative lesions. Another method of identification is a fluorescent antibody test, but this technique has the same limitations of a 5 micron tissue sample size, as does the Warthin-Starry stain. Additionally, the monoclonal antibody is not commercially available.

PCR on postmortem mucosal samples was compared to the standard postmortem diagnostic tool, the Warthin-Starry stain, for detection of *L. intracellularis* to evaluate the performance of PCR on mucosa. In this study, PCR on mucosal scrapings was more sensitive and specific for *L. intracellularis* than the Warthin-Starry stain. There are several factors that influence the sensitivity of PCR testing. PCR may be detecting the organism prior to lesion development or when bacterial cell integrity is lost. Additionally, the PCR preparation represents a larger portion of the ileum, thus increasing the sensitivity, whereas a histopathological section is representative of only a 5 micron cross-section. The IFAT test is specific for *L. intracellularis* and revealed organisms that were not detected by the Warthin-Starry stain. Due to the close correlation with traditional diagnostic techniques, PCR should be considered a useful tool for the detection of *L. intracellularis* in intestinal mucosa samples.

As summarized in Table 2, 16 of the samples were positive by PCR, Warthin-Starry and IFAT evaluation. Six samples were PCR positive, Warthin-Starry negative and IFAT positive. These samples could have lacked intact organisms necessary for positive Warthin-Starry test. Four samples were PCR positive but negative on evaluation with the Warthin-Starry and immunofluorescent stains. PCR evaluates a greater quantity of intestinal tissue, detects the organisms prior to lesion development and does not require intact bacteria. Additionally, in these cases the intestines may not have been evaluated during the necropsy process, such as in an uncomplicated pneumonia case. The remaining nine samples were negative by all three tests, but were diagnosed as PPE cases by the VDL pathologist on the basis of lesions seen on the necropsy examination.
The samples as described in more detail in Table 4, the Warthin-Starry stain failed to detect 6 out of 22 (27%) samples that were IFAT positive and 10 out of 26 (38%) samples that were PCR positive (Obs. 17-26). Ten positive PCR samples were not originally examined for lesions or organisms and were not clinically identified as PPE cases during the diagnostic procedure. On retrospective histological evaluation, eight of these ten had negative Warthin-Starry stains (Obs. 19-26). On the remaining two samples, organisms were present by the Warthin-Starry stain (Obs. 5 and 6). Additionally, there were two samples, positive by PCR, which were examined for the presence of organisms by the Warthin-Starry stain (Obs. 17 and 18). One of these two samples (Obs. 18) was diagnosed positive based on the supporting lesion, and the other sample (Obs. 17) was diagnosed as negative. On reexamination of these two samples during the study, both were negative for the presence of the \textit{L. intracellularis} by the Warthin-Starry stain. Furthermore, there were nine samples (Obs. 27-35) that were diagnosed by the pathologist as PPE on the basis of suspect gross or microscopic lesions, but there was no confirmation of organisms during the study by PCR, IFAT or Warthin-Starry. Hence, an inappropriate diagnosis could result when it is based on a lesion of enteritis rather than the presence of the organism.

Utilizing any pig submitted to the ISU VDL allowed a non-selective examination of ileal samples. Although the number of positive samples was small, it does indicate the various age groups harbor \textit{L. intracellularis}. The organism was present in 26 of 621 (4.2%) of the ISU VDL cases included in the survey (Table 3). The ages of the pigs that were positive ranged from two weeks of age to mature sows, but the majority of the positive samples were from nursery and grower-finisher pigs, 23 out of 346 samples from those age groups (6.6%). If the cases involved in the study were subclinical, the organism was being detected before clinical manifestations of this syndrome occurred.

The type of screening information provided by PCR analysis could lead to more timely antibiotic intervention and greater control of the infection. The importance of finding the organism in mature and neonate animals suggests the possibility of dams shedding with subsequent infection of the piglets. The organisms may reside in the mucosa subclinically.
with clinical disease occurring when pigs are moved to the nursery or grower-finisher unit resulting in a reduction of weight gain and alteration in feed efficiency.

A three site investigation was conducted in which PCR was used on pooled fecal samples (floor and rectal) to determine infected groups of pigs. The survey was done with commercial swine herds with a history of clinical PPE in the preceding twelve months. There was a history of medicated feed usage in all groups. PCR detectable shedding of *L. intracellularis* in the presence and absence of clinical disease was evident among these pig groups. There was detectable shedding in 4% to 32% of the individually sampled pigs in the pens with positive pooled floor samples. The pens housed between 5-25 pigs ranging from 10 to 24 weeks of age. Thus, not all exposed pigs were shedding but there is the likely possibility that this study also supported the theory that pooled rectal samples or pen floor samples can be utilized to detect shedding of *L. intracellularis*.

In another study a multiplex PCR (M-PCR) assay was evaluated for the detection of *L. intracellularis, Serpulina hyodysenteriae* and *Salmonella* species. The amplified sequences of *Salmonella, invE* and *invA*, are conserved throughout *S. typhimurium, S. choleraesuis, S. derby, S. brandenburg, and S. cubana* species. The veterinary diagnostic laboratories at the University of Nebraska-Lincoln and the University of Minnesota, livestock auction markets, and experimental pigs were the sources of specimens (mucosal scrapings and feces). The sensitivity and specificity for the multiplex assay was tested by using spiked cultures of varying concentrations of bacteria. Positive and negative M-PCR results were compared to the culture and histopathology results for the three organisms. All of the results correlated with the conventional diagnostic procedures except for one specimen which was M-PCR positive for a *Salmonella invA* sequence and the culture was negative. One advantage of the PCR test is that live organisms are not required. A multiplex PCR assay can provide the diagnostic rule-outs in a timely fashion and perhaps more economically than traditional diagnostic methods.

The impact of PPE could be better understood if fecal monitoring indicated the colonization status of *L. intracellularis*, but this is difficult to accomplish when shedding is
sporadic and when low numbers of bacteria are being shed. In some production units, *L. intracellularis* may be present but never produce clinical disease. An antemortem test is desirable for detection of an organism before it causes a clinical problem.

This study provides additional evidence that PCR is a sensitive diagnostic tool for confirming the presence of *L. intracellularis* in mucosal samples. The use of PCR as a monitoring and diagnostic tool could also assist in management of PPE in swine herds; however, the application as an antemortem tool is limited at this time because it is considered too laborious and expensive for routine diagnostic evaluation. In selected situations, it may be justified because of a higher degree of sensitivity and specificity; additionally, it is a rapid procedure that can be applied to batch testing of samples. PCR as a diagnostic tool to confirm the presence of *L. intracellularis* has the potential to be valuable to some producers.

**Acknowledgments**

This study was supported by a Merck Scholarship Grant. The PCR technology was contributed by Boehringer Ingelheim / NOBL Laboratories, Inc. We would like to thank the ISU VDL pathologists and bacteriology technicians for collecting and handling samples.

**References**


Table 1. Distribution of Samples by Age or Weight of Pigs

<table>
<thead>
<tr>
<th>Category</th>
<th>Age</th>
<th>Weight (lb.)</th>
<th># Sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>0-3 wk</td>
<td>&lt;10</td>
<td>258</td>
</tr>
<tr>
<td>Nursery</td>
<td>4-8 wk</td>
<td>10-50</td>
<td>130</td>
</tr>
<tr>
<td>Grower-Finisher</td>
<td>9-24 wk</td>
<td>51-240</td>
<td>216</td>
</tr>
<tr>
<td>Mature</td>
<td>&gt;25 wk</td>
<td>&gt;250</td>
<td>11</td>
</tr>
<tr>
<td>No Information Given</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2. Summary of Polymerase Chain Reaction (PCR), Warthin-Starry Stain (WS), and Immunofluorescent Antibody Test (IFAT) Results

<table>
<thead>
<tr>
<th>PCR</th>
<th>WS</th>
<th>IFAT</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>-</td>
<td>ND*</td>
<td>ND*</td>
<td>586</td>
</tr>
</tbody>
</table>

*No histopathology done
Table 3. Distribution of PCR Positive Samples By Age Category

<table>
<thead>
<tr>
<th>Classification</th>
<th>Number of Positives in Category/Total in Category (%)</th>
<th>Number of Positives in Category/Total Positives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>1/258 (.38)</td>
<td>1/26 (3.84)</td>
</tr>
<tr>
<td>Nursery</td>
<td>11/130 (8.46)</td>
<td>11/26 (42.31)</td>
</tr>
<tr>
<td>Grower-Finisher</td>
<td>12/216 (5.56)</td>
<td>12/26 (46.15)</td>
</tr>
<tr>
<td>Mature</td>
<td>2/11 (18.18)</td>
<td>2/26 (7.69)</td>
</tr>
<tr>
<td>Not Available</td>
<td>0/6 (0.00)</td>
<td>0/26 (0.00)</td>
</tr>
<tr>
<td>Total</td>
<td>26/621 (4.19)</td>
<td>26/26 (100.00)</td>
</tr>
</tbody>
</table>

Table 4. Age Classification, Results of PCR, WS, and IFAT Tests, and Diagnosis

<table>
<thead>
<tr>
<th>Obs.</th>
<th>Age</th>
<th>PCR</th>
<th>WS</th>
<th>IFAT</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>Porcine Proliferative Enteritis (PPE)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td>PPE; No significant bacteria</td>
</tr>
<tr>
<td>3</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td>Necrotizing ileitis, PPE Salmonellosis</td>
</tr>
<tr>
<td>4</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td>Bronchopneumonia, <em>Pasteurella multocida</em>, <em>Actinomyces pyogenes</em></td>
</tr>
<tr>
<td>5</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>Polyserositis, no evidence of PPE</td>
</tr>
<tr>
<td>6</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td>PPE; No significant bacteria</td>
</tr>
<tr>
<td>7</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>PPE; No significant bacteria</td>
</tr>
</tbody>
</table>

h G/F = Grower-Finisher
i M = Mature
j N = Nursery
<table>
<thead>
<tr>
<th>Obs.</th>
<th>Age</th>
<th>PCR</th>
<th>WS</th>
<th>IFAT</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td>PPE; No significant bacteria</td>
</tr>
<tr>
<td>9</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td>PPE; No significant bacteria</td>
</tr>
<tr>
<td>10</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>PPE; No significant bacteria</td>
</tr>
<tr>
<td>11</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>PPE; Colitis; <em>Salmonella choleraesuis</em></td>
</tr>
<tr>
<td>12</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>PPE</td>
</tr>
<tr>
<td>13</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>PPE; Smooth <em>Escherichia coli</em></td>
</tr>
<tr>
<td>14</td>
<td>N</td>
<td>(-)</td>
<td></td>
<td></td>
<td>Mucohemorrhagic colitis, lesions suggestive of PPE; No significant bacteria</td>
</tr>
<tr>
<td>15</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td>PPE</td>
</tr>
<tr>
<td>16</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td>PPE</td>
</tr>
<tr>
<td>17</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>Necrotic enteritis, Salmonellosis, Porcine Respiratory and Reproductive Syndrome, <em>Actinobacillus pleuropneumoniae</em></td>
</tr>
<tr>
<td>18</td>
<td>N</td>
<td></td>
<td>(-)</td>
<td></td>
<td>PPE; No significant bacteria</td>
</tr>
<tr>
<td>19</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td>Pneumonia, <em>Salmonella</em></td>
</tr>
<tr>
<td>20</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>Pneumonia; <em>A. pleuropneumoniae, P. multocida</em>, PRRS</td>
</tr>
<tr>
<td>21</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>Idiopathic hemorrhagic bowel syndrome</td>
</tr>
<tr>
<td>22</td>
<td>NEO</td>
<td></td>
<td></td>
<td></td>
<td>PRRS, Streptococcus meningitis, <em>E.coli</em>, Rota virus enteritis</td>
</tr>
<tr>
<td>23</td>
<td>G/F</td>
<td></td>
<td></td>
<td></td>
<td>Bronchopneumonia; <em>A. pleuropneumoniae</em></td>
</tr>
</tbody>
</table>

*NEO = Neonate*
Table 4. (continued)

<table>
<thead>
<tr>
<th>Obs.</th>
<th>Age</th>
<th>PCR</th>
<th>WS</th>
<th>IFAT</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>G/F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><em>A. pyog Lexington</em></td>
</tr>
<tr>
<td>25</td>
<td>N</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Septicemia; Meningitis; TGE villous damage</td>
</tr>
<tr>
<td>26</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><em>Mycoplasma hyopneumoniae</em></td>
</tr>
<tr>
<td>27</td>
<td>G/F</td>
<td>-</td>
<td>(-)</td>
<td>-</td>
<td>Hemorrhagic bowel syndrome, mild proliferative enteritis</td>
</tr>
<tr>
<td>28</td>
<td>N</td>
<td>-</td>
<td>(-)</td>
<td>-</td>
<td>Colitis; lesions suggestive of PPE</td>
</tr>
<tr>
<td>29</td>
<td>G/F</td>
<td>-</td>
<td>(-)</td>
<td>-</td>
<td>Suppurative bronchopneumonia, <em>M. hyopneumoniae</em>; Gastric ulcers; PPE</td>
</tr>
<tr>
<td>30</td>
<td>N</td>
<td>-</td>
<td>(-)</td>
<td>-</td>
<td>Pneumonia; <em>M. hyopneumoniae</em>, PRRS, <em>A. pleuropneumoniae</em>; Salmonellosis</td>
</tr>
<tr>
<td>31</td>
<td>G/F</td>
<td>-</td>
<td>(-)</td>
<td>-</td>
<td>Salmonellosis, PPE</td>
</tr>
<tr>
<td>32</td>
<td>G/F</td>
<td>-</td>
<td>(-)</td>
<td>-</td>
<td>PPE; Smooth <em>E.coli</em></td>
</tr>
<tr>
<td>33</td>
<td>na¹</td>
<td>-</td>
<td>(-)</td>
<td>-</td>
<td>PPE; <em>E.coli</em></td>
</tr>
<tr>
<td>34</td>
<td>G/F</td>
<td>-</td>
<td>(-)</td>
<td>-</td>
<td>Polyserositis; PPE</td>
</tr>
<tr>
<td>35</td>
<td>N</td>
<td>-</td>
<td>(-)</td>
<td>-</td>
<td>Salmonellosis; PPE</td>
</tr>
</tbody>
</table>

The parenthesis indicate a change in from the initial result of the test.

Results in parenthesis in the PCR column indicate the result of the secondary amplification of the initial PCR product.

Results in parenthesis in the WS column indicate the result upon the re-evaluation of the ISU VDL slide.

¹ na = not available
Figure 1. A 4% agarose gel demonstrates the 319 bp band that results from the PCR amplification of the DNA samples. In lanes 2 and 20 are weak positive pure culture controls; lanes 17 and 35 are strong positive pure culture controls. Lanes 16 and 34 are the negative controls. Lanes 1, 18, 19, and 36 contain the DNA ladder. The 26 positive PCR samples fill in the remaining lanes.
Sources and Manufacturers


A LAWSONIA INTRACELLULARIS TRANSMISSION STUDY USING A PURE CULTURE INOCULATED SEEDER-PIG SENTINEL MODEL

A paper to be submitted to Swine Health and Production

DM Jordan, JP Knittel\textsuperscript{a}, EM Groth\textsuperscript{b}, KJ Schwartz\textsuperscript{c}, MB Roof\textsuperscript{d}, DJ Larson\textsuperscript{e}, LJ Hoffman\textsuperscript{f}

Summary

Objective

To further validate PCR for the detection of \textit{L. intracellularis} in feces, by comparing colonization, fecal shedding and seroconversion patterns in a group of pigs inoculated with a pure-culture of \textit{L. intracellularis} and a sentinel group of pigs exposed to the inoculated group. By mixing naive pigs with infected pigs, it could be determined if PCR-detectable shedding correlated with transmission.

Methods

Four trials were conducted where conventional pigs were divided into three groups. One group was inoculated with a pure culture of \textit{Lawsonia intracellularis}, one group served as sentinels, and one group was designated as controls. Seven to 13 days after the first group was inoculated, the pigs were individually paired with a sentinel pig for five to seven days. Fecal monitoring for PCR detectable shedding was conducted on a regular basis. Serum samples were collected and titers for \textit{L. intracellularis} were determined by indirect fluorescent antibody techniques (IFAT). At the end of the pairing period, the inoculated group was necropsied. The sentinel and control pigs were necropsied 7 to 14 days following

\textsuperscript{a} Boehringer Ingelheim / NOBL Laboratories, Inc.; Project Leader
\textsuperscript{b} Iowa State University; Veterinary Student
\textsuperscript{c} Iowa State University; Veterinary Diagnostician
\textsuperscript{d} Boehringer Ingelheim / NOBL Laboratories, Inc.; Director of Research and Development
\textsuperscript{e} Iowa State University; Associate Professor, Veterinary Diagnostics Production Animal Medicine
\textsuperscript{f} Iowa State University; Professor, Veterinary Diagnostics Production Animal Medicine
the end of the pairing period. The intestinal tracts were evaluated grossly and microscopically for lesions. PCR was performed on intestinal mucosal scrapings and feces. Warthin-Starry and fluorescent antibody staining was done to assess colonization by \textit{L.intracellularis}.

\textbf{Results}

Two trials yielded typical gross lesions of porcine proliferative enteropathy in both the inoculated and sentinel groups. One trial yielded microscopic lesions and transmission to sentinel pigs. In the last trial, none of the pigs were colonized with \textit{L.intracellularis}. PCR results detected \textit{L.intracellularis} being shed in the feces which revealed a cyclical pattern of shedding. Seroconversion occurred in a predictable manner in the trials when maternal antibody was not present.

\textbf{Implications}

Fecal shedding of \textit{L.intracellularis} is intermittent. Cyclical patterns of fecal shedding were observed which did not coincide with clinical diarrhea. PCR-detectable shedding was observed when there were no gross lesions at necropsy. When there were gross proliferative lesions, colonization by \textit{L.intracellularis} was not always confirmed. Infective dose may be low, especially when major risk factors exist. Serconversion can occur without clinical disease or accompanying lesions. Maternal antibody may be protective in pigs less than four weeks old. PCR and serologic information are valuable tools when used on a herd basis.

\textbf{Introduction}

\textit{Lawsonia intracellularis} is the causative agent of porcine proliferative enteropathy (PPE). Enteric disease manifestations of PPE are most common in grower-finisher pigs. Risk factors such as high health status, temperature fluctuations, relocation, co-mingling, and poor sanitation are associated with outbreaks of PPE.

There are several studies evaluating the polymerase chain reaction assay for its usefulness to detect \textit{L. intracellularis} in feces and intestinal mucosal scrapings. Experimental
and field studies were designed to determine the specificity and sensitivity of the test as well as to show the incidence of the organism.1-6

The purpose of this study was to further validate PCR for the detection of *L. intracellularis* in feces, by comparing colonization, fecal shedding, and seroconversion patterns in pigs inoculated with a pure-culture of *L. intracellularis* and in sentinel pigs. By mixing naive pigs with infected pigs, it could be determined if PCR-detectable shedding correlated with transmission.

**General Materials and Methods**

Four trials were conducted for the purpose of demonstrating transmission of *L. intracellularis* from inoculated to naive sentinel pigs and assessing PCR as a method for detecting the organism.

*Pigs*: Preliminary screening of conventionally raised pigs from a herd without a history of PPE was done to assess the *L. intracellularis* infection status of the pigs prior to the beginning of the trials. Fecal swabs were collected and tested by PCR to detect *L. intracellularis*. Serum for the detection of antibodies to *L. intracellularis* was also collected; positive samples were attributable to maternal antibody.

The pigs were procured and randomly assigned to one of three groups: inoculated, sentinel, control. The inoculated group was intragastrically dosed after fasting 25 hours with a pure culture of *L. intracellularis*. Dexamethasone (8mg) was given to the inoculated group on Days 0, 2, 4, and 6 to suppress the immune system and enhance colonization of the organism. The other two groups were sham-inoculated with uninfected tissue culture. Clinical signs were assessed and fecal swabs were collected for PCR evaluation on alternate days. Individual pig weights were obtained biweekly and sera were collected weekly to determine antibody titers. Each inoculated pig was paired with a naive sentinel in a new pen when fecal shedding was detected by PCR. If no *L. intracellularis* was detected, pairing occurred by 13 days post-inoculation. The pigs remained paired for seven days. During this period, physical condition scores were assigned and fecal swabs were collected on a daily basis for each pig. After seven days of co-mingling, the inoculated pigs were necropsied for
evidence of infection. The sentinel and control pigs were necropsied between seven and fourteen days after the end of co-mingling.

**Clinical Scoring:** Clinical scores were recorded on a regular basis as described. The parameters evaluated included fecal consistency, behavior, general body condition, and hair coat. The criteria used are outlined in Table 1.

**Inoculum:** The pure culture inoculum was prepared and quantitated as previously described.9

**Necropsy:** At the designated times, the pigs were euthanized by electrocution. Gross lesions were recorded and samples of lesions were collected in 10% buffered neutral formalin for histopathology. If no gross lesions were present, the terminal ileum was collected for evaluation.

**Polymerase Chain Reaction:** The DNA extraction from the mucosal samples and fecal swabs was accomplished with a commercial guanidine thiocyanate nucleic acid extraction kit. The extracted DNA was subjected to PCR following a previously described protocol using commercial reagents. The PCR products were evaluated via electrophoresis on a 4% agarose gel. The DNA was stained by ethidium bromide and visualized under ultraviolet light. Positive *L. intracellularis* samples yielded a 319-bp band.7

**Histopathology:** Formalin-fixed tissues were processed routinely and evaluated histologically. Slides were stained with hematoxylin and eosin (H & E) and with the Warthin-Starry silver stain. The H & E slides were evaluated for lesions and the Warthin-Starry slides were designated as either positive or negative for the presence of curved rod-shaped organisms in the apical cytoplasm of crypt enterocytes.

**Indirect Fluorescent Antibody Test (IFAT):** An indirect immunofluorescent antibody test was conducted on the formalin-fixed tissue sections. Mouse-derived monoclonal IgG specific for *L. intracellularis* was used as described previously. The slides were evaluated

---


with a fluorescent microscope for the presence of curved intracellular organisms in the crypt enterocytes and were scored either positive or negative.

**Serology:** Serum antibody titers for *L. intracellularis* were evaluated using a recently developed qualitative IFAT serology test by Boerhinger Ingelheim / NOBL Laboratories, Inc.9

**Trial 1**

**Materials and Methods**

In Trial 1 there were 15 pigs housed in one room: 6 inoculated pigs, 6 sentinel pigs, and 3 control pigs; each group was maintained in a polytub (refer to Figure 5). One outside-room control was used. Sixty-two ml of inoculum provided a total dose of $10^5$ organisms per pig. The pigs were inoculated at 31 days of age. The pigs were paired 13 days post-inoculation. One inoculated pig died on Day 19 and was necropsied on Day 20. The remaining inoculated pigs were necropsied on Day 21 post-inoculation. One sentinel pig died on Day 27 and another on Day 28. The sentinels and in-room controls were necropsied one week after the end of co-mingling, Day 28 of the trial. The one outside-room control was necropsied at 33 days after the end of Trial 1. All fecal swabs were evaluated for *L. intracellularis* by PCR, and serum antibody levels were obtained using the IFAT.

**Results**

Six days after the pigs were paired, one inoculated pig died of hemorrhagic PPE which was confirmed by necropsy and histopathology. Two sentinel pigs died of hemorrhagic PPE on days 27 and 28 of the trial (days 14 and 15 after pairing).

Gross lesions typical of PPE were seen at necropsy in all of the inoculated pigs and all of the sentinel pigs. The hemorrhagic form occurred in 50% of the inoculated pigs and 17% of the sentinel pigs. None of the control pigs had typical gross lesions in the intestines. Intracellular organisms typical of *L. intracellularis* were observed in all of the inoculated and sentinel groups and in two of the three in-room control pigs by Warthin-Starry staining. IFAT results correlated 100% with the Warthin-Starry stain results. PCR conducted on ileal...
mucosal scrapings revealed 50% positive animals in the inoculated group, 83% in the sentinel group, and 66% in the in-room control group. However, of those in the inoculated group that had negative ileal scrapings by PCR, 2 of 3 had colonic mucosal scrapings that were PCR positive and also presented with the hemorrhagic form. In the sentinel group, the pig that was PCR negative from ileal scrapings had a positive jejunal scraping and also presented with PHE. Fecal shedding as detected by PCR occurred in 100% of the challenge and sentinel animals during the trial and one of the three (33%) in-room control pigs near the end of the trial. Figure 1 represents the pattern of shedding in Trial 1 pigs. Serologically, 100% of the inoculated pigs seroconverted within the 28 day study 50% seroconversion in the sentinels within 15 days of exposure. No seroconversion occurred in those pigs which were in-room controls (Figure 2). When the outside control pig was necropsied on Day 62 there were no gross lesions, no histopathologic lesions, nor evidence of organisms by Warthin-Starry or IFAT. PCR tests were negative on both mucosal scrapings and fecal swabs, nor was seroconversion detected.

Discussion

In Trial 1 there was a high percentage of infected animals in the inoculated and sentinel groups. As demonstrated in Figure 1, fecal shedding began at 8 days post exposure for inoculated animals and at day 16 for sentinel pigs which correlates to three days after pairing with inoculated pig partners. One in-room control pig began shedding on Day 24. It is speculated that this pig was infected inadvertently during the commingle period of the other pigs via aerosolization of fecal matter during cleaning or fecal contamination of the environment of the control pigs. In Figure 2, seroconversion correlated with detectable fecal shedding beginning at 14 days post exposure with 100% seroconversion at 22 days post exposure in the inoculated group. The sentinel group began seroconverting at 15 days after mingling with inoculated pigs. In Trial 1 there were differences in severity of both hemorrhagic and nonhemorrhagic forms of clinical disease following pure culture inoculation. Litter and individual pig variation and environmental stresses may have contributed to these differences.
Trial 2

Materials and Methods

Colonization, shedding, transmission, and seroconversion, were successfully demonstrated in Trial 1 so Trial 2 was designed to repeat Trial 1 with a larger number of pigs. The pigs for Trial 2 were housed in separate pens in the same room as Trial 1 before its completion. There were 30 pigs introduced into the room on Day 16 of Trial 1 as follows: 12 inoculated pigs, 12 sentinel pigs, and 6 control pigs. Six outside control pigs were housed separately. Each pig received 70 ml of inoculum intragastrically equal to $10^6$ bacteria per pig at 32 days of age. The pigs were paired 9 days post-inoculation when fecal shedding was detected in the inoculated group. The inoculated group was necropsied seven days later. The sentinels and both control groups were necropsied two weeks after the end of co-mingling (Day 33 of Trial 2). PCR evaluation for detection of *L. intracellularis* was conducted on all swabs collected.

Results

No mortality occurred in Trial 2, but gross lesions typical of PPE were observed in 92% of the inoculated pigs, 67% of the sentinels and 17% of the in-room control pigs. Warthin-Starry staining confirmed intracellular organisms in 83% of the inoculated pigs, 50% of the sentinel pigs and 17% of the in-room control pigs. Ninety-two percent of the inoculated pigs, 50% of the sentinel pigs and 17% of the in-room control pigs were positive by IFAT. PCR results for *L. intracellularis* on ileal mucosal scrapings were 92% positive in the inoculated group, 58% positive in the sentinel group, and 33% positive in the in-room control pigs. PCR detected fecal shedding of *L. intracellularis* in 92% of the inoculated pigs, 58% of the sentinel pigs and 67% of the in-room control pigs. Figure 4 represents the pattern of detectable shedding in Trial 2 pigs. Serologic tests showed that 100% of the inoculated pigs and in-room control pigs seroconverted, as well as 83% of the sentinels (Figure 5). The six outside control pigs were negative for the all of the above parameters.
Discussion

In Trial 2, lesions and organisms were present in all but one pig of the inoculated group, but the negative pig did seroconvert. The sentinel pigs did not develop lesions, colonization, or seroconvert in a predictable fashion as did the pigs in Trial 1. One room housing may be responsible for the potential opportunity for exposure to infective material to all pigs. It is believed that the Trial 2 pigs were exposed during the end of Trial 1. The evidence of early infection of the pigs introduced into the room because of inadvertent transmission of infective material from the infected Trial 1 pigs. As can be seen in the diagram of the room (Figure 6), the in-room control pigs were situated behind the cages of the paired pigs from Trial 1 that were actively shedding the bacteria. Fecal material could have been sprayed into the area of these control animals. Likewise, the sentinels of Trial 2 could have also been infected during this period by sprayed infectious material or by personnel who inadvertently transferred infectious material to the pigs and their environment. As seen in Figure 4, fecal shedding as detected by PCR indicated that infection occurred at unexpected times when compared to Figure 1 of Trial 1. The sentinel group, as well as the in-room control group, was actively shedding prior to co-mingling. Seroconversion followed the same trend with all of the pigs responding in a similar fashion (Figure 5). An explanation for differences in infectivity and shedding responses in Trial 2 may be an immunologic response elicited by a low dose exposure to the actively shedding inoculated and sentinel animals from Trial 1.

Trial 3

Materials and Methods

Trial 3 was conducted because of the inconsistency in results due to inadvertent contamination problems in Trial 2. There were 24 pigs introduced to Iowa State University Veterinary Medical Research Institute facilities at 22 days of age: 9 inoculated pigs, 9 sentinel pigs, and 6 control pigs. Each pig received $10^5$ bacteria in 15 ml intragastrically at 23 days of age. The pigs were paired 10 days post-inoculation. The inoculated pigs were
necropsied on Day 15. The sentinels and control pigs were necropsied 13 days after the end of co-mingling (Day 28). No PCR was done on the fecal swabs collected.

Results

There were no death losses among Trial 3 pigs. None of the pigs demonstrated gross lesions typical of PPE. Histologically, 11% of the inoculated pigs, 44% of the sentinels and 33% of the control pigs had microscopic lesions of mild enterocyte hyperplasia. Results by Warthin-Starry staining revealed intracellular organisms in 22% of the inoculated pigs but none in the sentinel and control pigs. There were no organisms detected by IFAT or PCR testing in any of the test groups. Due to the lack of evidence of *L. intracellularis* in the tissue, PCR was not conducted on fecal swabs. None of the pigs seroconverted.

Discussion

Minimal infection occurred in Trial 3. One inoculated pig out of nine (11%) showed evidence of colonization by intracellular organisms were present on the Warthin-Starry stain, but bacteria were not confirmed by IFAT. Mild hyperplastic lesions were evident in 11% and 44% of the inoculated and sentinel groups, respectively. There was no seroconversion in any of the Trial 3 pigs. These pigs were younger at the time of inoculation, 23 days old. Maternal antibody was present at the time of inoculation and may have played a role in preventing colonization in this trial (Figure 7). An additional factor which may have affected the colonization rate could include the physiologic environment of the stomach due to age and diet which may be different than a 31 day old pig’s gastric environment.

Trial 4

Materials and Methods

Trial 4 was performed as a final attempt to reproduce the results of Trial 1. Fifteen pigs were procured at 22 days of age and were divided into the following groups: 6 inoculates, 6 sentinels, and 3 controls. Each pig received $10^6$ bacteria in 12 ml intragastrically at 29 days of age. The pigs were paired 10 days post-inoculation. The
inoculated pigs were necropsied on Day 17. The sentinels and control groups were
necropsied 14 days after co-mingling on Day 31 of the trial. Fecal swabs were evaluated by
PCR on Days 10, 17, 22 and 31 of the study.

**Results**

There was no mortality, but there were clinical indications of enteric disease by
diarrheic stools in 50% of the inoculates and 33% on the sentinels. All of the pigs, inoculates
and sentinels, demonstrated gross lesions typical of PPE. In the inoculated group 67% of the
pigs had mild lesions while 33% of pigs had moderate lesions. Eighty-three percent of the
sentinel pigs had mild lesions, and 17% had moderate lesions. In this group 67% of the
intestinal sections were edematous. Histologically, only one sentinel pig demonstrated
enterocyte hyperplasia; Warthin-Starry staining also confirmed intracellular organisms in this
pig. When IFAT was performed on tissues, there was one positive pig in the each of the
inoculated and sentinel groups (17%). PCR tests on the ileal mucosa of two sentinel pigs
(33%) were positive; one being the same pig that was positive on IFAT. No *L. intracellularis*
was detected when PCR was conducted on fecal swabs. In the control pigs, there were no
gross or histologic lesions, nor any positive fecal samples by PCR. Seroconversion was not
demonstrated in any of the pigs (Figure 8).

**Discussion**

The results of Trial 4 reinforce the hypothesis that gross intestinal thickening is not
always attributable to *L. intracellularis* but could be due to other insult, infection, or edema.
Mild to moderate gross proliferative lesions were detected in 100% of the pigs in the
inoculated and sentinel groups, but only 19% were confirmed positive for *L. intracellularis.*
The pigs in which *L. intracellularis* was detected were #59 (inoculate), #61 (sentinel), and
#67 (sentinel). This creates an interesting scenario. The inoculated pig, #59, was paired with
the sentinel pig #60. The sentinels, #61 and #67 were paired with inoculated pigs #62 and
#66, respectively. Pigs #60, #62, #66 did not demonstrate any evidence of colonization by
*L. intracellularis.* With these results, assuming no cross contamination with fecal material
from pig #59, minimal colonization is necessary for shedding to occur. Even if contamination occurred, minimal fecal material transfer would be involved, further indicating the ease and risk potential for small amounts of fecal material to be considered an infective dose. Fecal swabs analyzed by PCR were negative; however, PCR was not conducted on all fecal swabs that were collected. The possibility exists that PCR is not sensitive enough to detect very low numbers of bacteria. Intermittent shedding is also common, therefore, some swabs may be truly negative for *L. intracellularis*.

In Trial 4 no seroconversion occurred after exposure. Maternal antibody present at the time of inoculation could have interfered with the colonization in this trial as in Trial 3. Alternatively, colonization may have been minimal and would not elicit a strong immune response or the response was not detectable at the times samples were procured.

**Conclusions**

The seeder-pig-sentinel model is a useful model to create a natural infection. Fecal shedding of the organism as detected by PCR is intermittent, as is the diarrhea observed clinically. An example of the cyclic nature is depicted in Figures 9 and 10 for the inoculated group of Trials 1 and 2. The first peak of elevated fecal scores is believed to be attributable to environmental stresses, but the later data suggests that the diarrhea follows after shedding has begun. This may be a significant observation. If shedding occurs prior to clinical signs, it may be too late to prevent transmission. If the early diarrhea was attributable to *L. intracellularis* infection, detectable shedding seems to follow the diarrhea by a few days. Not enough information is available to demonstrate a true correlation between onset of shedding and diarrhea. A correlation between these two parameters could be important to the industry for determining when testing and intervention would be most effective.

In Trials 1 and 2 several major risk factors were present such as temperature fluctuations, co-mingling, and suboptimal sanitation. This may have had a compounding effect on the transmission study. The infective dose seems to be low and easily transmitted throughout the room despite precautions. In Trial 4 sentinel pigs were colonized even though their inoculated penmate did not show evidence of colonization or shedding, further
suggesting a low dose requirement for this disease. There seems to be no need for direct pig to pig contact for transmission. This can be significant in production units because there seems to be a definite risk of transmission to neighboring pens. During these studies a naive pig source was used. The outside-room controls of Trials 1 and 2 remained negative throughout both studies, as did the controls in Trials 3 and 4. This study shows that the organism is transmitted easily within an enclosed environment and that strict biosecurity is necessary to control *L. intracellularis*.

There was no evidence of colonization by *L. intracellularis* in any of the pigs in Trial 3, but there was microscopic hyperplasia of enterocytes. Gross lesions were present in Trial 4 but colonization was only evident in a total of three pigs; two by PCR, 2 by IFAT, and 1 by Warthin-Starry histopathological staining, reemphasizing the need for organism identification to make the diagnosis of PPE. The colonization and positive results in pigs at necropsy in Trial 4 occurred without any indication of infection in the live animals.

There was no seroconversion of pigs in Trials 3 and 4 whereas in Trial 1 and 2 there was seroconversion. The main difference between the pigs’ serologic status was the presence of antibody titers to *L. intracellularis* at the time of inoculation of pigs in Trial 3 and 4 which were not present in pigs from Trials 1 and 2. The pigs utilized were seven and two days younger than the pigs in Trial 1. The age of susceptibility and maternal immunity may be significant factors as well as individual and litter variation. The pigs in Trial 3 and 4 were also from different sows than those in Trials 1 and 2, but all were from the same closed herd. No studies have been done to determine the protective nature of the maternal antibody or if there is participation of mucosal immunity. When colonization did not occur to a high degree in Trials 3 and 4 with maternal antibody present, the inference could be made that maternal antibody is protective. However, maternal antibody was only present until around four weeks of age. This window of susceptibility occurred at a stressful point in the production cycle which may alter vulnerability to infection. Other factors that may influence the colonization are physiologic factors, especially stomach pH and diet, that may differ significantly in a 20 day old pig and a 30 day old pig.
When the serologic data is compared to shedding as in Trials 1 and 2, it is seen that seroconversion may occur without PCR detectable shedding. This suggests that low levels of bacteria may elicit seroconversion without the degree of colonization that is necessary to elicit shedding or clinical signs.

In a management scheme where PPE is a problem or control measures need to be evaluated, PCR and IFAT serology may be useful tools. Serum collected at various phases of production can be evaluated for antibodies against *L. intracellularis* to give an indication of exposure and risk. With this information preventive measures can be implemented.

To optimally utilize PCR, several animals which are representative of the herd or group (clinical and nonclinical pigs), should be evaluated. Fecal swabs and pen floor samples could be pooled and evaluated. If samples are positive, it would be concluded that shedding animals are present. Results of these studies support the hypotheses that fecal monitoring and serology are valid tools for herd surveillance in appropriate situations.

**Implications**

- Shedding of *L. intracellularis* is intermittent.
- Gross lesions aren’t necessary for shedding.
- Gross proliferative lesions are not always due to *L. intracellularis*.
- Identification of *L. intracellularis* is necessary for the accurate diagnosis of PPE.
- Infective dose may be low.
- Serconversion occurs without clinical disease or accompanying lesions.
- Maternal antibody may be protective.
- PCR and serologic information can be used on a herd basis.

**Acknowledgments**

These trials fulfilled the objectives of a grant awarded from the National Pork Producers Council. Additional support was provided by the Iowa Healthy Livestock Initiative and Boehringer Ingelheim / NOBL Laboratories, Inc. The authors would like to acknowledge the veterinary students who assisted with the animal studies: Eva Groth, Brian Huedepohl,
and Dale Faulhaber. A special thanks is extended to Jeff Knittel of Boehringer Ingelheim / NOBL Laboratories, Inc. for the provision of the *L. intracellularis* culture. Additionally, Boehringer Ingelheim / NOBL Laboratories, Inc. unselfishly shared their laboratory space and equipment for testing.

References


Table 1. Clinical Scoring Criteria

<table>
<thead>
<tr>
<th>Pig ID</th>
<th>Date</th>
<th>Stool</th>
<th>Behavior</th>
<th>Body Condition</th>
<th>Hair Coat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td></td>
</tr>
<tr>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td></td>
</tr>
<tr>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td></td>
</tr>
<tr>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td></td>
</tr>
<tr>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td></td>
</tr>
</tbody>
</table>

- **Stool Consistency**
  - 1 Firm
  - 2 Semi firm
  - 3 Diarrhea
  - 4 Dead

- **Behavior**
  - 1 Active / Alert
  - 2 Lethargic
  - 3 Huddling
  - 4 Dead

- **Body Condition**
  - 1 Full Abdomen
  - 2 Gaunt
  - 3 Thin
  - 4 Dead

- **Hair Coat**
  - 1 Smooth
  - 2 Rough
  - 3 Bristled
  - 4 Dead
Figure 1. Trial 1: Detection of *L. intracellularis* in Feces by PCR
Figure 2. Trial 1: Antibody Response to *L. intracellularis*
Figure 3. Trial 1: Comparison of Detectable Fecal Shedding by PCR with Antibody Response to *L. intracellularis*
Figure 4. Trial 2: Detection of *L. intracellularis* in Feces by PCR
Figure 5. Trial 2: Antibody Response to *L. intracellularis*
Figure 6: Room Design For Trials 1 and 2
Figure 7. Trial 3: Antibody Response to *L. intracellularis*
Figure 8. Trial 4: Antibody Response to *L. intracellularis*
Figure 9. Trial 1: Fecal Shedding as Detected by PCR vs Fecal Scores in Inoculates
Figure 10. Trial 2: Fecal Shedding as Detected by PCR vs Fecal Scores in Inoculates
GENERAL CONCLUSIONS

Veterinarians are noting an increase in various forms of porcine proliferative enteritis in high-health status, SEW, and MEW herds. Although many diseases are being controlled by all-in all-out management, many minor or secondary pathogens are coming to the forefront. Herd prevalence for PPE caused by *Lawsonia intracellularis* is not known. For accurate diagnosis of PPE, *L. intracellularis* must be identified rather than relying on lesions alone.

In an attempt to estimate the occurrence of PPE in Iowa, a study was conducted on all porcine ilea (regardless of age or health status) submitted to the Iowa State University Veterinary Diagnostic Laboratory between December 1, 1994, and June 30, 1995. It was shown that 4% of the pigs tested were positive for *L. intracellularis*. Positive PCR results for *L. intracellularis* were distributed over all age groups, but were most common in the nursery and grower-finisher categories. *L. intracellularis* was also noted in neonate animals, which suggests the possibility of sows shedding bacteria with subsequent infection of the piglets. The organisms may reside in the mucosa with no clinical disease present until pigs enter the nursery or grower-finisher unit where the greatest impact of the disease is noted with reduction in rate of gain and feed efficiency. The PCR results were compared to the traditional method of detection, Warthin-Starry staining of tissue sections, as well as the fluorescent antibody test. When the results of these comparisons were compiled, it appeared that PCR was more sensitive than H&E and Warthin-Starry histopathologic stains, and the fluorescent antibody was more specific than the Warthin-Starry stain. This study could have been improved by testing feces simultaneously with the mucosa samples for comparative purposes.

There are two antemortem tests currently available for detecting *L. intracellularis* on a limited basis from a private diagnostic laboratory. PCR on feces and serum antibody testing. A disadvantage of the PCR test is that false negatives can occur because the organism is shed intermittently. The serologic test has been developed which detects IgG antibody against *L. intracellularis*. Serologic information, if collected on a herd basis and interpreted correctly,
can provide insights for intervention strategies at points of exposure in the production system. Improvements to these antemortem diagnostic tools will facilitate investigations for prevalence, treatment effectiveness, and economic impact of PPE.

A second study was designed to determine if there is a correlation between initiation and duration of fecal shedding and seroconversion. A seeder-pig-sentinel model utilizing a pure culture inoculum to create a natural infection in a naive pig was used to minimize confounding factors in the expression of disease. This model was also used to determine if PCR on feces could detect transmissible shedding of the organism. It was found to be useful for producing infection and studying detectable shedding patterns. Fecal shedding of the organism as detected by PCR was intermittent and somewhat cyclical, as was clinical diarrhea. Detectable shedding and diarrhea did not occur simultaneously. This observation may be important with respect to treatment and prevention protocols. During the trials it was apparent that the disease could be transmitted among animals with fecal contamination of pens, which alludes to the importance of careful management in production facilities.

When seroconversion was studied in these trials, there was an indication that maternal antibody may represent protection from disease. This is an aspect of the disease that needs further study. In the pigs used in two of the trials, there were detectable antibody titers to *L. intracellularis* at the time of exposure. These animals did not have clinical signs or pathologic lesions of PPE. It was beyond the scope of the study to postpone necropsy of the pigs to determine if the animals would succumb to the disease at a later time. Additionally, seroconversion was observed in the absence of clinical disease or detectable shedding, which may imply that low numbers of bacteria can elicit an immune response.

In conclusion, the impact of PPE could be better understood if fecal monitoring by PCR consistently reflected the colonization status of *L. intracellularis*, but this is difficult to accomplish when shedding is sporadic and when low numbers of bacteria are being shed. For monitoring on a herd basis, fecal PCR and serologic testing can be utilized.
ACKNOWLEDGMENTS

For the successful completion of my Master of Science degree with my Doctor of Veterinary Medicine degree I have many people to acknowledge. The parties directly involved are those at Iowa State University College of Veterinary Medicine and Boehringer Ingelheim / NOBL Laboratories, Inc. At Iowa State the pathologists in the diagnostic laboratory were instrumental in the collection of samples for my first study. Dr. Kent Schwartz provided the diagnostic pathology as well as moral support for all of the studies. The Veterinary Diagnostic Bacteriology and Histopathology Laboratories are also commended for their role throughout my research. The researchers at Boehringer Ingelheim / NOBL Laboratories, Inc. were wonderful with which to work; they provided me with insight, support, and expertise. A special thanks is extended to Gloria Jordan for helping organize my data and graphs. My committee was essential in the direction and planning of my degree. Dr. Lorraine Hoffman was a tremendous asset during my professional and graduate education. Her commitment to my degree programs and goals is greatly appreciated.

I would also like to recognize my very encouraging veterinary classmates who have been important in my life these last four years. My family, especially my husband, Daniel, has been immensely supportive through my college career. The encouragement from my immediate family as well as my extended family in the Collins - Maxwell community has been wonderful.