PORCINE PROLIFERATIVE ENTEROPATHY (ILEITIS): DIAGNOSTICS AND IMMUNITY

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Porcine proliferative enteropathy (PPE) is a common infectious disease that affects weaned pigs of various ages. The disease, commonly called ileitis, occurs worldwide and produces variable clinical manifestations including a chronic form, called porcine intestinal adenomatosis (PIA), and an acute form, called proliferative haemorrhagic enteropathy (PHE).

The chronic form is common in pigs between 6 to 20 weeks old and results in poor growth, uneven weight gain and, as a result, delay to market. Transient diarrhoea is common, but not always present.

PHE, the acute form of PPE, affects older pigs from 4 to 12 months of age. Affected pigs are pale and their faeces are black or bloody. They may develop massive intestinal haemorrhage and die suddenly.

Both the chronic and acute forms of PPE have unique histologic features, such as the proliferation of intestinal epithelial cells; within these cells, there are many intracellular curved organisms.

The etiologic agent of PPE is *Lawsonia intracellularis*, an obligate intracellular bacterium. Identification of this intracellular organism has facilitated the development of specific assays that can be used to diagnose the disease and detect immunity in affected pigs.

DIAGNOSIS OF PPE

Clinical signs.

PPE is difficult to diagnose because the clinical signs are non-specific or even lacking. Dramatic weight variation between pigs of the same age is a hallmark of chronic PPE. Overall poor performance, gauntness or soft-to-watery stools may occur. In the acute form of the disease, an occasional pig may die suddenly, followed by the sporadic occurrence of pigs with bloody diarrhoea.

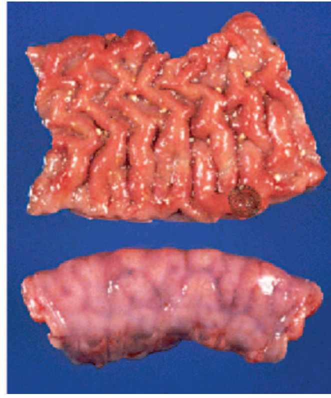
Postmortem diagnosis of PPE.

Conventionally, the diagnosis of PPE has been confirmed based on gross lesions seen at necropsy and on microscopic tissue examination. Although severe PPE lesions can be diagnosed at necropsy, the more common moderate to mild lesions can be difficult to diagnose and are often overlooked.

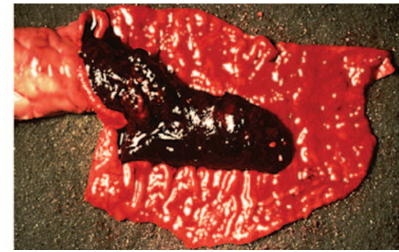
Histopathological examination of tissues can confirm a diagnosis of PPE. The two most specific and sensitive methods for diagnosis are polymerase chain reaction (PCR) of ileal mucosa using specific primers and immuno-histochemistry of tissue sections using a specific monoclonal antibody.

Gross lesions.

Gross lesions vary depending on the clinical manifestation of the disease and may appear as haemorrhagic or chronic. If they are noted, they



Gross pathology of the small intestine from a pig with the chronic form of proliferative enteropathy, porcine intestinal adenomatosis (PIA). Note the thickened, ridged mucosa.



Gross pathology of the small intestine from a pig with the acute form of proliferative enteropathy, proliferative haemorrhagic enteropathy (PHE). Note the thickened mucosa and blood clot in the lumen.

are most commonly seen in the ileum, near the ileal-cecal junction, and appear as a thickening of the mucosa. More chronic disease results in necrotic enteritis or a thickening of the outer muscular layer. In PHE, a large amount of undigested blood is seen in the small intestinal lumen.

Histology.

Histologically, a diagnosis of PPE can be made by demonstrating the presence of proliferative enterocytes on routine H&E staining, but evaluation of proliferation may be subjective and only cases with typical severe enterocyte proliferation can be diagnosed. Staining of histologic sections using a silver stain reveals the presence of numerous intracellular organisms with a characteristic curved shape. These organisms are typically located within the apical cytoplasm of the crypt epithelial cells. However, this method is not specific for *L. intracellularis* and cannot always detect the organism in necrotic debris or in autolyzed tissue.

Immunohistochemistry.

More specific identification of *L. intracellularis* can be achieved by immunohistochemistry staining of fixed tissues. This technique is more sensitive than the silver stain because it reveals organisms within macrophages of the lamina propria during recovery from PPE. In addition, extracellular *L. intracellularis* can be identified either in exudate or necrotic debris in superficial mucosa. A specific monoclonal antibody, which is not readily available, is required for this test. In a study comparing diagnostic methods, immunohistochemistry staining detected nearly twice as many pigs with PPE lesions as did silver staining of formalinized tissues.

PCR of ileal mucosa.

If immunohistochemistry is not available, specific identification of *L. intracellularis* in the intestine can be achieved by PCR of the ileal mucosa. This technique is readily available in most diagnostic laboratories and, when applied to ileal mucosa rather than faeces, is as specific as immunohistochemistry.

Antemortem diagnosis of PPE.

Several techniques have been described for the detection of *L. intracellularis* in live pigs. Culture of the organism from faeces is not an option because the agent is an obligate intracellular bacterium. Other methods of antemortem diagnosis include detection of *L. intracellularis*

"…immunohistochemistry stainin

in faeces by PCR or indirect antibody staining, and serological assays for *L. intracellularis* antibodies using either an indirect fluorescent antibody (IFA) test or an immunoperoxidase monolayer assay (IPMA).

Cultivation of L. intracellularis.

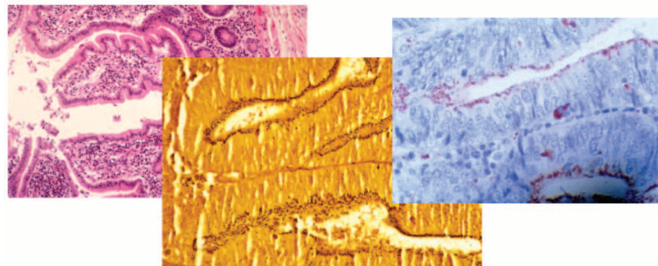
Cultivation of *L. intracellularis* can be achieved in intestinal cell lines, but with difficulty. The organism must be purified from a naturally contaminated intestine; it requires a specific microaerophilic environment and invades only actively dividing cells. Since little or no cytopathic effect can be seen, special stains are required to monitor *L. intracellularis* growth. Infected pigs do shed *L. intracellularis* in the faeces beginning about 1 week post-challenge, but diagnosis of PPE by cultivation of the organism from faeces is not practical or even possible. Consequently, routine sensitivity testing of isolates is not possible. Because of the difficulty in culturing *L. intracellularis* from intestines, there are only about 12 isolates worldwide that have been maintained *in vitro* in cell cultures. Several of these isolates have been tested for their susceptibilities to various antimicrobial agents.

PCR assays.

Several PCR assays have been developed for detection of *L. intracellularis* in faeces. The sensitivity and specificity of the PCR technique in faecal samples has been evaluated in many reports, which show variable sensitivity (depending upon the comparisons made) and consistently high specificity (about 97%). Sensitivity is affected by sample quality and the presence of inhibitory factors in faeces. PCR appears to reliably demonstrate *L. intracellularis* in the faeces of clinically affected pigs, but is not sensitive enough to routinely detect the organism in faeces from subclinically affected animals.

Indirect antibody technique.

Demonstration of *L. intracellularis* in faeces can also be accomplished using a specific mono-clonal antibody in an indirect antibody staining technique. It is a standard laboratory technique that is not affected by PCR inhibitors. However, expertise is required to evaluate the results and the availability of the technique is limited by the need for a specific monoclonal antibody. Also, like PCR, the technique lacks sensitivity for diagnosing subclinically affected animals.



Typical view of pig intestinal sections stained with H&E stain, Warthin-Starry silver stain and immuno-histochemical stain with Lawsonia*-specific antibodies, respectively. Note the proliferation of the enterocytes in the H&E stain, the presence of numerous curve-shaped, black-stained intracellular organisms in the silver stain and specifically-stained antigen (red) in the immunohistochemical stain.*

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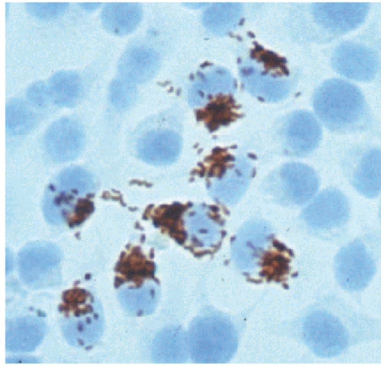

Lawsonia intracellularis *organisms grown in McCoy cell culture. Numerous intracellular bacteria in the cytoplasm are stained (red) using an immunoperoxidase technique incorporating* L. intra-cellularis-specific polyclonal antibody.

Serological assays.

Serological assays are based on the detection of *L. intracellularis* antibodies in serum. Current methods used for serological diagnosis of PPE employ *L. intracellularis* cultured in enterocytes or a preparation of *L. intracellularis* on slides as the antigen. These assays are specific because cell cultures or slides are examined micro-scopically and specifically stained bacteria can be distinguished from any background. Staining of bacteria is either by a fluorescent (IFA) or peroxidase-labeled (IPMA) secondary antibody. Serology results were similar when using the IFA or IPMA tests and both were found to be equally sensitive and specific in evaluation of experimentally infected animals. Serologic assays have proved to be useful for routine diagnosis of PPE, although the humoral immune response of *L. intracellularis*-infected pigs is often weak and short-lived. Furthermore, serology provides only historical information on exposure of the animal to *L. intracellularis*. In the PIA form of PPE, infection may incubate several weeks before eliciting positive serum responses.

COURSE OF THE DISEASE

The application of *L. intracellularis* diagnostics during challenge trials has provided insight into the typical course of chronic PPE. The incubation period is 7 to14 days with early lesions appearing in the terminal ileum. Faecal shedding begins about 7 days post-challenge and animals seroconvert about 14 days post-challenge. The disease peak is about 21 days after infection. Clinical signs decrease and proliferative lesions resolve after 28 days of infection, resulting in a 2-week delay in obtaining market weight.

L. intracellularis is present in proliferating crypt cells, but is gone when the cells return to normal maturity. Faecal shedding and seropositivity cease at some point after resolution of the disease. Less is known about the acute, PHE, form of PPE other than that it has a similar incubation period, but with acute clinical signs.

SUMMARY OF PPE DIAGNOSTICS

The most sensitive tests for diagnosing PPE are mucosal PCR and tissue immunohistochemistry, but both require necropsy to perform. The antemortem methods are less accurate, with serology being most sensitive. The routine postmortem tests, gross exam, tissue H&E and silver stain are the least sensitive.

PCR detection of *L. intracellularis* in faeces is an adequate test for PPE when necropsy is not possible. The indirect antibody detection of *L. intracellularis* in faeces appears more sensitive, but specificity is not known. Serology is a sensitive test for detecting pigs that have been exposed to *L. intracellularis*.

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Serological and faecal shedding assays may now be used for cross-sectional or serial disease profiling to determine the timing of pig exposure to *L. intracellularis*. These assays may also aid in the evaluation of preventive and control strategies through the timing of medication or other intervention strategies for PPE.

IMMUNITY

There is little understanding of the host immune response against *L. intracellularis*. Information from studies focusing on the humoral and, in particular, cell-mediated immune response against *L. intracellularis* infections may be used to evaluate the effectiveness of prevention and control strategies.

Humoral antibody response.

Challenge trials.

Much of the information we have on the humoral immune response of pigs has been obtained through challenge trials using mucosal homogenates from pigs affected by PPE. In these trials, weaned pigs were challenged orally at 5 weeks of age with mucosal homogenate from a PPE-affected pig. The animals were necropsied 3 to 4 weeks post-challenge and generally all challenged pigs had histological lesions of PPE at that time. Gross lesions were reported in about 80%. IgG titers of 1:30 to *L. intracellularis* appeared about 2 weeks after challenge. Up to 90% of the pigs became positive 3 weeks after challenge, with 5% showing titers of 1:480 or greater. At 4 weeks post-challenge, titers began to decay and a decreased percentage of the pigs were positive.

Natural PIA outbreaks.

Seroconversion in growing pigs during a natural field outbreak of PIA showed a slightly different pattern. Some nursing piglets showed *L. intracellularis* IgG titers of 1:30, which decayed within 3 to 4 weeks. In grow to finish pigs, titers of only 1:30 to 1:60 were detected beginning at about 12 weeks of age. The percent of pigs affected peaked around 24 weeks of age and then declined. For individual pigs, titers decayed within 3 to 4 weeks. Mild or no clinical signs of PPE were noted in these outbreaks.

Natural PHE outbreaks.

In contrast, high humoral IgG titers specific for *L. intracellularis* were obtained from pigs affected with PHE in a natural outbreak. Affected animals showed IgG titers as high as 1:1920. Titers decayed by about half every 3 weeks after peak and eventually were undetectable. Some sows that had survived PHE had detectable (1:30) IgG titers to *L. intracellularis* at farrowing, suggesting that sows may confer passive immunity to piglets. Effectiveness and length of protection provided by passive immunity has not yet been established.

Sample area of a 96-well plate used in the IPMA serology test. Red-colored wells indicate positively stained Lawsonia *organisms in the well, and therefore, IgG antibody in the serum applied to those wells. Insert shows a microscopic view of the cell-cultured, intracellular* Lawsonia *stained in the IPMA test.*

Cell-mediated immune response.

Intracellular organisms usually stimulate a cell-mediated immune response. The ELISPOT-T cell assay detects *L. intracellularis*-specific secretion of interferon-gamma by memory or activated T-lymphocytes. The assay is evaluated based on the number of T-lymphocytes specifically activated against *Lawsonia* antigens.

Recently, the ELISPOT assay was evaluated for detection of production of interferon-gamma in the leukocytes of pigs experimentally challenged with *L. intracellularis*. Results of the assay parallel the humoral response in that specific interferon-gamma production begins about 2 weeks after challenge, peaks at 3 weeks and then begins to decay, though less slowly than the humoral response. However, the test may not be useful diagnostically since it requires *L. intracellularis* antigen and fresh viable lymphocytes for testing.

Experimentally challenged pigs also elicit a delayed-type hypersensitivity (DTH) reaction. DTH reactions were evaluated 24 and 48 hours after intradermal injection of different concentrations of *L. intracellularis* antigen 20 days after challenge. Challenged animals showed a dose-dependent DTH reaction that was more evident 24 hours after injection. Cell-mediated immunity and both local (*Lawsonia*-specific IgA in the ileum) and systemic humoral immune responses were detected in experimentally infected pigs.

APPLICATIONS

The availability of antemortem diagnostic tools coupled with an understanding of the immune response of pigs affected with PPE will foster development of more strategic methods of PPE prevention and control. Serology profiles can be correlated with clinical disease or faecal shedding of *L. intracellularis* on pig farms and the results can be used to confirm the diagnosis and determine the timing of intervention strategies.

Infected pigs demonstrate both a humoral and cell-mediated immune response to *L. intracellularis*. Furthermore, infection results in immunity to subsequent challenge. Therefore, strategies allowing infection of pigs with *L. intracellularis* followed by antimicrobial intervention to prevent clinical disease may enable development of a protective immune response.

As we learn more about PPE through accurate diagnostics, we may be able to use the results of immunity studies to develop more strategic methods of management and medication to control, prevent and possibly eradicate PPE.

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