Tests to diagnose subclinical ileitis

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Ileitis is a common infectious disease affecting weaned animals of various ages and species. The disease occurs worldwide in many animal species but is of special economic importance in the swine industry. There are several different manifestations of ileitis in pigs, including an acute form, proliferative haemorrhagic enteropathy (PHE) and a chronic form, porcine intestinal adenomatosis (PIA).

A subclinical form of ileitis is more recently recognised in which L. intracellularis infections occur with no apparent clinical signs of the disease. All these forms of ileitis share unique histological features, including various degrees of proliferation of the immature epithelial cells of the intestinal crypts, causing a thickening of the mucosa of the small and, sometimes, large intestine.

Within these proliferating enterocytes are many intracellular, curved bacteria, identified as Lawsonia intracellularis. Identification and characterisation of L. intracellularis as the etiology of ileitis has facilitated development and refinement of specific assays for diagnosis of the disease in affected pigs.

The subclinical form of ileitis occurs in the early growing period but animals have no overt clinical signs. Clinically, diarrhoea is not apparent, but a decreased growth rate results in lowered performance, as evidenced by reduced average daily gain, poor feed conversion, and increased variation in weights within a group.

Gross lesions typical of ileitis may be present, but are not easily recognised. Classic histological lesions (for example, proliferation of enterocytes with large numbers of intracellular lawsonia within cells) are present, but of insufficient severity to cause observable clinical signs.

Past diagnostic tools

For many years, ileitis was diagnosed based on history, clinical signs, and necropsy with demonstration of typical gross pathology of affected animals.

Clinically, even the PIA and PHE forms of ileitis are difficult to diagnose because the signs are non-specific. In addition, the various clinical forms may mimic other enteric diseases, including salmonellosis, swine dysentery, colibacillosis, colonic spirochetosis, transmissible gastroenteritis, rotavirus infection, haemorrhagic bowel syndrome and porcine circovirus 2 infection.

In acute PHE, affected pigs may be pale and their faeces black or bloody. An occasional pig may develop intestinal haemorrhage and die suddenly, followed by sporadic occurrences of pigs with bloody diarrhoea. Chronic PIA is characterised by poor growth, uneven weight gain and a delay to market. Overall, poor performance, gauntness or soft to watery stools may occur.

Transient diarrhoea often occurs, but is not always present in this form of ileitis. The subclinical form of ileitis, though likely present, was rarely recognised.

Conventionally, a diagnosis of ileitis was confirmed by postmortem exam of gross lesions seen at necropsy and microscopic tissue examination (see photographs).

Gross lesions vary depending on the clinical manifestation of the disease and may appear as haemorrhagic or chronic. In PHE, there may be a large amount of undigested blood in the small intestinal lumen. Severe lesions of PIA are easily seen, however, the more common moderate to mild lesions may be hard to detect.

If noted, they are usually seen in the ileum near the ileal-caecal junction and appear as a mucosal thickening. More chronic disease results in necrotic enteritis or a thickening of the outer muscular layer. Gross lesions of subclinical ileitis can only be noted with experience, and then must be confirmed histologically.

Histologically, a diagnosis of ileitis was confirmed by demonstrating the presence of proliferative enterocytes on routine H and E staining, but evaluating proliferation may be subjective; only cases with the more severe enterocyte proliferation can be diagnosed. Staining of histological sections using a silver stain reveals numerous intracellular organisms with a characteristic curved shape, usually in 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 autolysed tissue.

A more specific identification of L. intracellularis is achieved by immunohistochemistry staining of fixed tissues, which is more sensitive than the silver stain because it shows organisms within mononuclear cells in the lamina propria during recovery from ileitis. In addition, extracellular L. intracellularis can be identified in exudate or necrotic debris in superficial mucosa.

In a comparative diagnostic study, immunohistochemistry staining detected nearly twice as many pigs with ileitis lesions compared to silver staining of formalin fixed tissues.

Nevertheless, this diagnostic method required necropsy of animals and so was not useful for herd studies, regardless of the clinical manifestation of ileitis present.

Present diagnostic tools

Currently, ante-mortem tests capable of detecting a specific serologic response to L. intracellularis or

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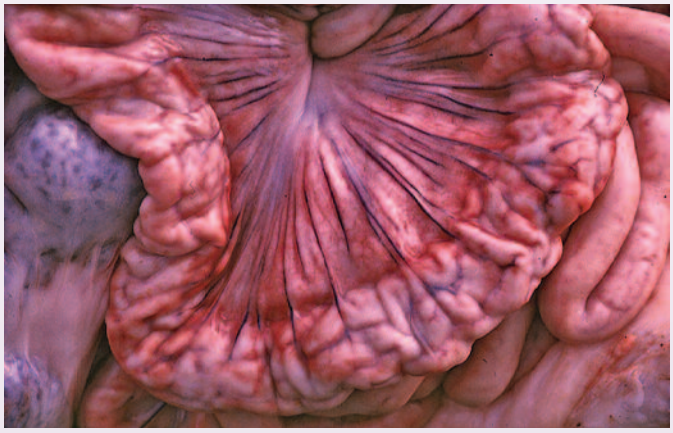
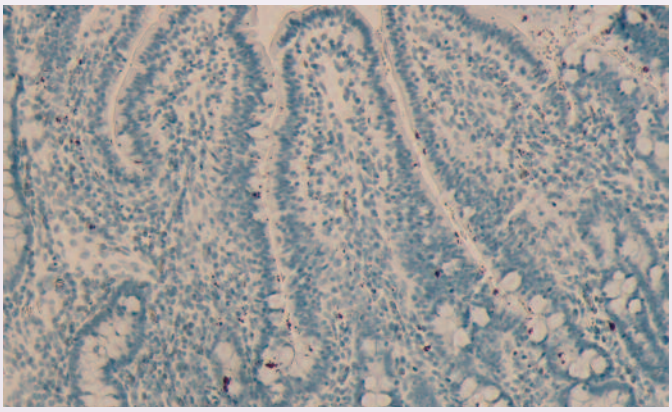
Gross lesions of a chronic Lawsonia intracellularis infection.



Immunohistochemistry staining of histopathological changes in the ileum tissue due to a sub-clinical infection with Lawsonia intracellularis.



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shedding of the organism in faeces are available in most countries.

These may be used to screen farms or herds for the mild clinical or subclinical outbreaks of ileitis.

With these ante-mortem tests, larger numbers of animals can be screened without the necessity of euthanasia.

These more sensitive and specific diagnostic measures are now enabling studies of prevalence of the disease in pig herds.

Several PCR assays have been developed that can detect L. intracellularis in faeces. The sensitivity and specificity of PCR in faecal samples have been evaluated in many reports, which show variable sensitivity (39-72%) and consistently high specificity (approaching 100%).

Sensitivity can be adversely affected by sample quality and the presence of inhibitory factors in faeces. It is important to incorporate a faecal DNA extraction procedure that results in a DNA template free of substances that inhibit the reaction. 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.

Pooling of faecal samples for PCR reduces the costs but decreases the sensitivity of the test. Two multiplex-PCR tests for detection of L. intracellularis, Brachyspira hyodysenteriae or B. pilosicoli or for detection of L. intracellularis, B. hyodysenteriae and salmonella, respectively, from pig intestinal specimens have also been developed.

These PCR tests offer the advantage of simultaneous screening and detection of DNA from several intestinal pathogens in a single reaction. However, they are also more technically complicated to perform.

Demonstration of L. intracellularis in faeces can also be done with a specific antibody in an indirect antibody staining technique or an immunological method using immunomagnetic separation and ATP bioluminescence.

However, expertise is required to evaluate the results and the availability of such techniques is limited by the need for a very high quality lawsonia specific antibody. Also, like PCR, these techniques may lack sensitivity for diagnosing subclinically affected animals.

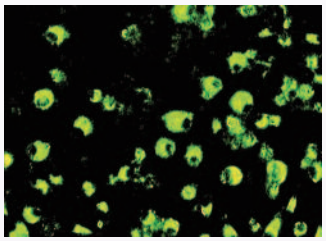
Current serological assays based on the detection of L. intracellularis antibodies in serum employ pure cultures of L. intracellularis, either used as a whole cell antigen on slides or plates or as a soluble antigen. Staining of bacteria is either by a fluorescent (IFA) or peroxidase labelled (IPMA) secondary antibody.

Recently, several groups have reported ELISA based methods for detecting humoral IgG to L. intracellularis. These ELISAs have the advantage of high throughput sample testing, automated reporting of results and less chance of bias in sample evaluation. They include an indirect LPS-ELISA, a sandwich ELISA with LPS as the antigen, a blocking ELISA, and a sonicated whole cell ELISA.

The blocking ELISA has become commercially available for screening herds worldwide. It is an indicator of herd exposure to L. intracellularis infection and has been shown to be as sensitive and specific as previous serology tests.

Agreements between tests vary and, unfortunately, all require culti-

Immunofluorescence assay (IFA) of Lawsonia intracellularis antibodies.



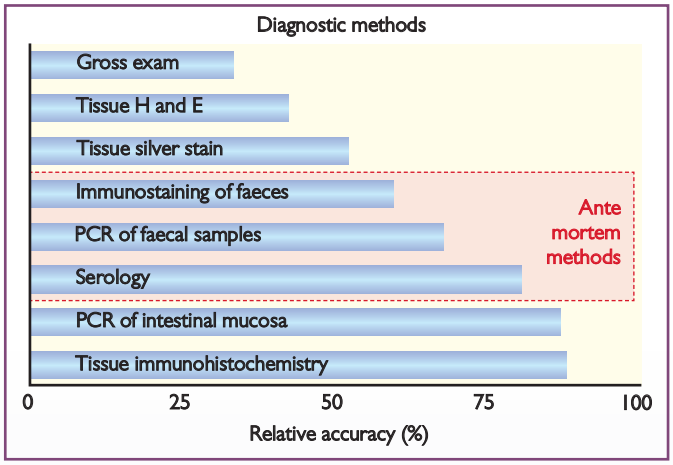


Fig. 1. Relative accuracy of diagnostic tests.

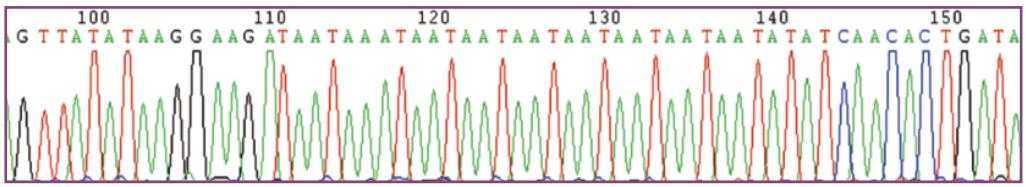


Fig. 2. Example of an area in the Lawsonia genome showing tandem repeats.

vation of lawsonia in vitro for antigen production.

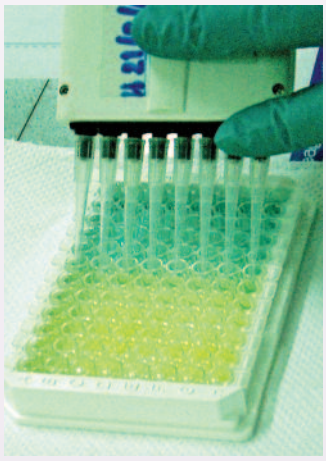
Serologic assays have proven useful for routine diagnosis and determination of prevalence of ileitis in pig herds worldwide (ranging from 60-90% in different countries), although the humoral immune responses of pigs with the PIA and, especially, the subclinical forms of PE are often weak and short lived.

Furthermore, serology provides only historical information on exposure of the animal to L. intracellularis. Its use is highly suited to cross-sectional and serial profiling studies of humoral immune response for implementation of control and prevention measures in herds in which ileitis is endemic.

Seroprofiling results will vary with any change in the immune status of the breeding herd or change in in-feed antimicrobial medication programmes in a group of pigs.

Present diagnostic testing

The challenge for diagnosis of ileitis in live animals is detection of subclinically and chronically affected animals. In order to confirm ileitis in a herd and associate it with a reduced performance problem, cross-sectional or, more preferably, serial faecal and serum samples should be tested.



Enzyme linked immunosorbent assay (ELISA) of Lawsonia intracellularis antibodies.

Faecal samples from at least 15 animals (with diarrhoea or runted, if available) should be collected from the rectum, kept on ice, and delivered to a laboratory for lawsonia PCR analysis.

If possible, two or three smaller pigs should be euthanised, evaluated grossly and samples sent for histology and immunohistochemistry for lawsonia. If serology testing is available, it should also be utilised as this technique is more sensitive than PCR of faeces, especially when identifying subclinically affected herds.

Furthermore, serologic profiling of herds can be used to better estimate the optimal time to intervene with a control programme. For seroprofiling, animals from five weeks of age to market age, with three to four week intervals between them, should be bled and tested for L. intracellularis IgG antibodies.

Detection of IgG in serum occurs about two weeks post infection and the duration lasts from three to 12 weeks after the first detection. This timeline may vary with the form of ileitis and the severity of the disease in the herd.

The severity of lesions appears to correlate with serum titres, and so very low titres would be detected in those pigs that are subclinically affected. Serum IgG titres decay by one-half titre every two to three weeks.

Gilts, affected with the PHE form, and five week old pigs infected with high doses of a pathogenic lawsonia, have shown high serum IgG titres that are still detectable up to 12 weeks after first detection.

Conversely, seropositivity in grow-finish pigs in field conditions is low (1 : 30 titre) to undetectable and lasts for only several weeks.

This window of seropositivity appears to be even shorter for subclinically affected pigs.

In the field, faecal shedding of lawsonia as evaluated by PCR, detects positive animals earlier in the course of the infection than does serology. Animals that are PCR positive in faecal samples may not have diarrhoea but are considered infected, shedding bacteria, and likely having reduced growth performance.

PCR positive and seronegative animals are either in an early stage of infection and have not yet had time to seroconvert or the level of infection was not sufficient to induce a detectable systemic humoral immune response.

Seropositive and faecal PCR negative animals may have been previously exposed to lawsonia but are not shedding bacteria anymore or detection of faecal shedding was limited by the low sensitivity of the PCR technique in faecal samples.

Therefore, in evaluating PCR shedding and seroprofile patterns in a herd, the percentage of animals that are seropositive and/or shedding lawsonia in the faeces and observation of poor growth performance must be evaluated as a whole.

Fig. 1 represents an overview of the currently available tests and their relative accuracy.

Future diagnostic tools

We have recently sequenced the entire genome of L. intracellularis. This information is now being mined for development of more sensitive and high throughput diagnostic test reagents for detection of ileitis and, especially, subclinical ileitis.

As there are very few laboratories worldwide that are able to grow lawsonia in sufficient numbers for use as antigen for serology testing, the cost in some areas is prohibitive. In the future, recombinant or synthesised immunogenic antigens may make serodiagnosis more available.

Two ELISA tests which utilise subunits of lawsonia have been reported, but are not yet routinely used for pigs.

A third ELISA, a blocking ELISA using whole bacteria, is presently commercially available in many parts of the world.

Recently developed real-time PCR methods offer the advantage over conventional PCR of the ability to quantitate L. intracellularis in samples as well as high throughput identification of positive samples. This ability to quantitate the lawsonia shedding will give us a better understanding of the dose effect of L. intracellularis in field cases as well as in challenge trials.

Little is known about the source or epidemiology of L. intracellularis in the field because of the lack of isolate differentiation techniques.

Due to the obligate intracellular nature of this organism, isolation and cultivation is extremely difficult, making the identification of L. intracellularis subtypes by traditional methods unfeasible.

L. intracellularis isolates from pigs, as well as from a variety of other animal species, are phenotypically indistinguishable and standard genomic subtyping methods have not proven to provide an acceptable level of discrimination for lawsonia isolates.

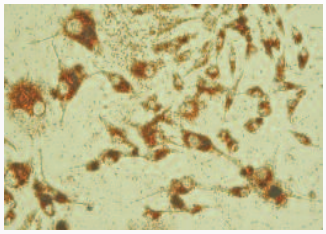
Although L. intracellularis if antigenic very conservative it has recently been shown that segments identified in the genome sequence of L. intracellularis contain markers with potential for demonstration of variation between lawsonia isolates.

The utility of this epidemiologic profiling method lies in the possibility for tracking and tracing different isolates of lawsonia obtained from different animal species, geographical locations, and field outbreaks of ileitis.

We have recently demonstrated that the L. intracellularis isolates in faecal samples from epidemiologically unrelated outbreaks had unique genetic profiles.

In contrast, L. intracellularis isolates from the same outbreak shared identical profiles.

Profiles remained identical within a herd regardless of the clinical form of ileitis documented, for example, Lawsonia isolates from the PIA, PHE, or subclinical forms of ileitis all shared the same profile within a herd.



Immunoperoxidase monolayer assay (IPMA) of Lawsonia intracellularis antibodies.

This leads to the conclusion that the phenotypic expression, for example, pathogenicity or immunogenicity of an isolate is not related to these non-coding parts of the genome, but is multi-factorial.

Much of the basic knowledge regarding lawsonia infection and transmission is presently unknown and could be clarified using this technique.

In the future, molecular typing may be employed along with faecal PCR in a high throughput diagnostic procedure to determine the source, as well as the presence and transmission route, of lawsonia isolates.

Summary

All forms of ileitis in pigs as well as other animal species are caused by the obligately intracellular L. intracellularis. Lawsonia is a unique bacterium, which causes an unusual pathology in infected animals, including proliferation of the infected mucosal epithelial cells of the intestine.

Growth performance of pigs subclinically infected with L. intracellularis is poor and these pigs shed the organism into the environment, infecting others in the herd, and resulting in poor growth performance.

Recent improvements in diagnostics for ileitis have allowed more accurate determination of the presence and prevalence of subclinical ileitis in these pigs. ■