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Ileitis: Evaluation and interpretation of serological results

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Introduction

Porcine proliferative enteropathy (PPE) or ileitis is a widespread enteric disease affecting the aboral small intestines and, sporadically, the large intestines of growing pigs. The epidemiological status of the swine population worldwide regarding PPE is poorly understood. The two ante-mortem methods for diagnosis of PPE available are PCR of fecal samples and serology. Serology is cheaper and more amenable to high throughputs.

Early serological tests, both IFAT (Lawson et al., 1988) and ELISA (Holyoake et al., 1994), developed to diagnose PPE utilized bacteria purified from affected pig intestines. Consequently, detected humoral response was weak and variable. Knittel et al. (1998), using an IFAT with pure culture of *L. intracellularis* in 96-well plates, demonstrated sensitivity of 90% in serological testing of experimentally infected pigs three weeks after challenge. In that study, they showed that IFAT serology was more sensitive than PCR in fecal samples, 90 and 39%, respectively. An impediment in performing this IFAT serology is that *L. intracellularis* is an obligate intracellular bacterium, thus, very difficult to grow *in vitro* for antigen production. In addition, some experience and expertise is necessary to prepare the IFAT plates.

As a result of the limitations mentioned above, little information is available in the literature about the level of humoral immune response in pigs naturally or experimentally infected with *L. intracellularis*. In one study, IgG could be first detected two weeks after challenge of five-week-old pigs with pure culture of *L. intracellularis*. Antibody levels (IgG) peaked around the end of the third week and then tended to drop (Knittel et al., 1998). No information is available about the duration of humoral immune response or passive immunity related to PPE.

The immunoperoxidase monolayer assay (IPMA) is a serological enzyme immunoassay test largely used for diagnosing viral infection, for instance porcine respiratory and reproductive syndrome (Nodelijk et al., 1996; Sorensen et al., 1998). *L. intracellularis*, as an obligate intracellular bacterium, infects cell line monolayers *in vitro* and proliferates in the cytoplasm of these cells. As a result, cell culture monolayers highly infected with *L. intracellularis* can be used in the same way as virus infected cell line monolayers to perform serology using the IPMA.

IPMA preparations can be examined using an inverted light microscope and the color reactions are stable for several months, thereby providing a lasting record of the results (Soliman et al., 1997). In contrast, IFAT preparations require a fluorescent microscope and can only be stored for a short time. In addition, *L. intracellularis* IPMA preparations are easier to interpret than fluorescent preparations. Consequently, the reliability of the IPMA is likely higher than that of IFAT.

This paper will show recent data from three different studies using the IPMA serologic method in experimentally and naturally infected pigs. The first study compares IPMA with the currently used serologic technique, IFAT. The second evaluates the specificity and sensitivity of IPMA. The third study shows the serologic follow-up of a breeding herd after an outbreak of acute ileitis and of the first offspring of some of the sows.

IFAT and IPMA comparison

Thirty five-week-old pigs weighing between 20 and 30 pounds were obtained from a herd with no history of PPE or any other confounding diseases. Twenty-eight pigs were inoculated with intestinal homogenate from PPE-diseased mucosa via stomach tube on days 0 and 1. The two remaining pigs (control) were allotted to a different pen separated by solid wood walls from the pens with challenged pigs. These two animals were inoculated with 25 ml of sucrose phosphate glutamate in the same manner used for challenged animals on days 0 and 1. Blood samples were collected from all animals at days 0, 7, 14, 22, and 28 post-inoculation. Serum samples were tested by IFAT (Knittel et al., 1998) and by IPMA (Guedes et al., 2000). All pigs were euthanized 28 days after inoculation. Samples of ileum were fixed in 10% neutral buffered formalin and processed routinely for histology. To confirm the infected status, one section was stained by immunohistochemistry (IHC). The concentration of positive antigen labeled was graded as follows:

- zero, no positive antigen labeled
- 1+, one isolated focal area of antigen labeled

- 2+, multifocal areas of antigen labeled
- 3+, majority of the mucosa had positive antigen labeled
- 4+, all of the mucosa had positive antigen labeled

The serology tests using both methods agreed in all samples, except on days 0 and 7 of one control animal (**Table 1**). This was probably due to maternal immunity and slightly higher sensitivity of the IPMA. One challenged animal was negative throughout the study in both tests; however, the IHC showed multifocal areas of antigen label in some mucosal glands. Two challenged animals were seropositive on days 14, 21, and 28 post-challenge in both tests, but did not have positive antigen label in any ileum sections. Possible explanations for these results are that these animals seroconverted without any lesion, the lesions were resolved before the day of euthanasia, or the lesions were restricted to the jejunum and

Table 1: Serology results on days 0, 7, 14, 22, and 28 post-challenge using IPMA (P) and IFAT (F), and IHC of ileum sections from samples collected 28 days after challenge.

Seq[1]	Pig #	Group[2]	Day 0		Day 7		Day 14		Day 22		Day 28		IHC
			P	F	P	F	P	F	P	F	P	F	
1	36	C	-	-	-	-	-	-	-	-	-	-	0
2	132	C	+	-	+	-	-	-	-	-	-	-	0
3	23	Ch	-	-	-	-	+	+	Died day 17				4+
4	47	Ch	-	-	-	-	-	-	Died day 21				3+
5	137	Ch	-	-	-	-	+	+	+	+	Died day 23		3+
6	158	Ch	-	-	-	-	-	-	+	+	Died day 24		4+
7	64	Ch	-	-	-	-	-	-	+	+	+	+	4+
8	13	Ch	-	-	-	-	-	-	+	+	+	+	3+
9	152	Ch	-	-	-	-	-	-	+	+	+	+	3+
10	35	Ch	-	-	-	-	-	-	+	+	+	+	3+
11	1	Ch	-	-	-	-	-	-	+	+	+	+	3+
12	149	Ch	-	-	-	-	-	-	-	-	+	+	3+
13	100	Ch	-	-	-	-	-	-	+	+	+	+	2+
14	81	Ch	-	-	-	-	-	-	+	+	+	+	2+
15	28	Ch	-	-	-	-	-	-	+	+	+	+	2+
16	55	Ch	-	-	-	-	-	-	+	+	+	+	2+
17	106	Ch	-	-	-	-	-	-	-	-	-	-	2+
18	98	Ch	-	-	-	-	-	-	+	+	+	+	1+
19	135	Ch	-	-	-	-	-	-	+	+	+	+	1+
22	24	Ch	-	-	-	-	-	-	+	+	+	+	1+
21	84	Ch	-	-	-	-	-	-	+	+	+	+	1+
22	115	Ch	-	-	-	-	-	-	+	+	+	+	1+
23	57	Ch	-	-	-	-	-	-	+	+	+	+	1+
24	172	Ch	-	-	-	-	-	-	+	+	+	+	1+
25	71	Ch	-	-	-	-	-	-	+	+	+	+	1+
26	34	Ch	-	-	-	-	-	-	+	+	+	+	1+
27	138	Ch	-	-	-	-	+	+	+	+	+	+	1+
28	113	Ch	-	-	-	-	+	+	+	+	+	+	1+
29	90	Ch	-	-	-	-	+	+	+	+	+	+	0
30	16	Ch	-	-	-	-	+	+	+	+	+	+	0

[1]Seq: Sequence of animals
[2]C: control group; Ch: challenged group

were not detected by IHC stain and examination of ileum sections.

In conclusion, IPMA and IFAT showed similar results in experimentally infected pigs; however, IPMA preparations do not require the use of a fluorescent microscope and can be stored for a long time.

IPMA validation

Based on the estimated sensitivity of the IPMA in the previous study (92.8%, 26 out of 28 infected pigs were seropositive) and an expected margin of error of 6.7%, the sample size of 120 five-week-old pigs, 60 pigs per group were used in this study. All animals were from a PPE-free farm. The animals were divided into two groups. Sixty pigs were transported to an isolated unit and inoculated with an intestinal homogenate from PPE-diseased mucosa via stomach tube. The remaining 60 animals were kept at the source farm as negative controls. Fecal samples from 40 animals (20 controls and 20 infected animals) were collected on day 0 to confirm the negative status of the herd. Fecal samples from all 120 animals were collected on day 21 and tested by PCR for *L. intracellularis* to assure that the infected group responded as expected to the challenge and the control group was still negative at the end of the experiment. Serum samples collected from all animals on days 0 and 21 were blindly tested using IPMA. The IPMA results were tested for sensitivity and specificity in a 2x2 table using the exposed and non-exposed status as "true conditions." Sensitivity and specificity were evaluated for different cut-off points (serum dilutions).

All serum samples collected on day 0 were negative by IPMA, as were the 40 fecal samples collected on day 0 and tested by PCR. Four of the 60 challenged pigs had to be humanely euthanized during the experiment due to poor clinical condition. All 60 fecal samples collected from the non-infected pigs on day 21 were negative by PCR. Forty-six of the 49 fecal samples collected from the challenged pigs on day 21 tested positive by PCR. The specificity and sensitivity results of the IPMA using different sera dilutions as cut-off points of 56 challenged and 60 non-infected pigs is shown in **Table 2**.

Based on the results, we conclude that the IPMA is a highly specific serologic test. The sensitivity of the dilution 1:15 is slightly higher than the dilution currently used as the cut-off point (1:30) but not significantly. The IPMA seems to be an appropriate diagnostic test for herd screening but not for diagnosing PPE on the individual level.

Serologic follow-up of a breeding herd after an outbreak

A recent outbreak of PHE in a breeding herd, previously PPE-free (naive gilts), was diagnosed by all available means (clinically, macroscopic and microscopic lesions, immunohistochemistry, and PCR). Thirty-six gilts, including 13 recovered animals, were bled three weeks after the beginning of the outbreak and then every three weeks until they became seronegative on two consecutive tests. Fourteen piglets from five gilts seropositive at farrowing and five piglets from two sows that remained seronegative throughout were bled once or twice at the farrowing house and then every three weeks until they reached market age. Fecal samples from these pigs were tested by PCR from seven weeks of age and then every three weeks until they reached market age.

Serological results from the gilts are summarized in **Figure 1**, and serological and fecal PCR results from the piglets are summarized in **Table 3**.

Gilts recovered from PHE had high serum antibody levels that lasted for up to three months in some animals. Piglets from sows that were seropositive at farrowing had detectable passive antibodies for up to five weeks of age. Some nursery pigs started shedding bacteria around seven weeks of age but antibody levels were not detected until 16 weeks of age.

General conclusions

- IFAT and IPMA show similar results.
- IPMA is a highly specific and fairly sensitive serological test for detecting exposure to *L.intracellularis*
- Pigs recovered from the acute form of PPE have high serum antibody levels that last for at least three months in some animals.
- Maternal immunity against *L. intracellularis* lasts for at least five weeks.
- Nursery pigs may start shedding *L. intracellularis* around seven weeks of age.
- Growing pigs start presenting low titers of antibodies against *L. intracellularis* around 16 weeks of age.

Table 2: Specificity and sensitivity of IPMA using different serum dilutions as cut-off points.

	Undiluted	**1:15**	**1:30**	**1:60**	**1:120**
Specificity (%)	5	100	100	100	100
Sensitivity (%)	92.6	90.7a	88.9ab	81.5ab	75.9b

[ab] Values within a row without a common small case letter differ ($P < 0.05$)

Figure 1: IPMA serum titers of gilts from a herd starting three weeks after a PHE outbreak.

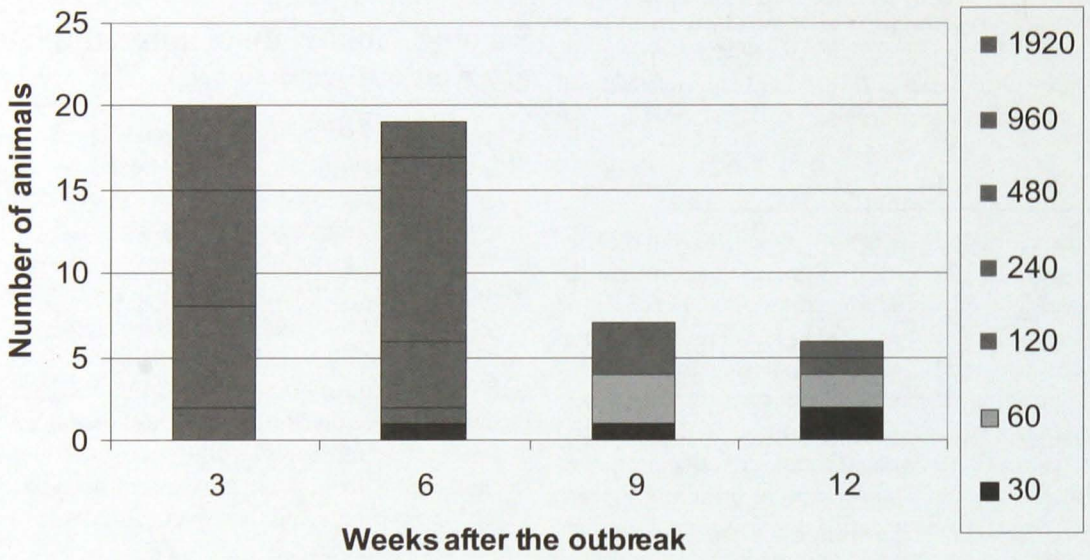

Table 3: IPMA serum titers from the first week of age until market with three-week-intervals between bleedings and PCR on fecal samples of those same piglets from seven weeks of age until market.

	Age (weeks)							
	1-2	**5**	**7**	**10**	**13**	**16**	**19**	**22**
Serum titers								
Negative	15	16	19	18	18	16	11	0
1:30	4	1	0	0	0	2	4	4
1:60	0	0	0	0	0	0	3	2
Total	19	19	19	18	18	18	18	6
Fecal samples								
PCR +	-	-	1	2	7	7	3	0
PCR -	-	-	18	16	11	11	15	6

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