

***Lawsonia intracellularis* ELISA: A New Test at the ISU-VDL**

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Ronaldo L. Magtoto, research associate;
John K. Johnson, clinician;
Sheela Ramamoorthy, clinician,
Department of Veterinary Diagnostic and
Production Animal Medicine
Iowa State University College of Veterinary Medicine

Summary and Implications

Porcine Proliferative Enteropathy (PPE) is a common disease of swine which is caused by the intracellular bacterium, *Lawsonia intracellularis*.

The performance of a recently available commercial ELISA for *L. intracellularis* was evaluated by comparison with an immuno-peroxidase monolayer assay (IPMA) and found to have at least 97% correlation. The same assay when conducted at different laboratories showed 100% agreement in results. Examination of serum samples from various cases submitted to the Iowa State University Veterinary Diagnostic laboratory indicated that 87% of the herds examined were sero-positive for *L. intracellularis*. Therefore, the Enzyme-Linked Immunosorbent Assay (ELISA) for *Lawsonia intracellularis* is a useful and reliable test which allows veterinary practitioners and producers to obtain same day results on swine serum samples submitted.

Introduction

The lesions Proliferative Enteropathies (PE) also known as *ileitis* were first described in pigs in Ames, IA by Biester and others in 1931. The cause of PE is *Lawsonia intracellularis*, an obligately intracellular bacterium. It grows in the cytoplasm of intestinal epithelial cells and causes the thickening of the mucosa and colon. Infection of pigs with this bacterium is consistently linked with the presence of proliferative lesions of the mucosa of the ileum and large intestine. The disease has a worldwide distribution and occurs commonly in all pig raising regions and in all farm management systems. Recent estimated losses due to *Lawsonia intracellularis* exceed \$121.00 USD per affected breeding pig which translates to an extra \$0.61 USD per growing pig.

Lawsonia intracellularis does not grow on conventional bacterial media and requires tissue culture under reduced oxygen tension. However, serological diagnosis of PPE is commonly achieved by means of tests such as indirect immunofluorescence assays (IFA) or immuno-peroxidase monolayer assays (IPMA), both of which involve cumbersome procedures to culture the organism *in-vitro*. ELISA tests are advantageous in that they

are easy to perform and do not require culture of the organism.

In this study we have evaluated the performance of a commercially available ELISA for the detection of antibody responses to *Lawsonia intracellularis*

Materials and Methods

ELISA: Kits manufactured by Synbiotics Europe SAS, were utilized following manufacturer's instructions. The optical density (OD) is measured at 450 nm in a microplate reader and the percent inhibition (PI) of the positive controls and test samples relative to the negative controls is calculated. Any serum sample presenting a PI of $\geq 30\%$ is considered positive. Any samples presenting a PI of $\leq 20\%$ are considered negative. Samples within 20-30% range are considered suspects.

Serum samples: A set of 40 serum samples out of which twenty were known negatives from uninfected controls and twenty were known positives from infected controls were provided by Boehringer Ingelheim Vetmedica, Inc. (BIVI). A total of 308 serum samples derived from eight different cases submitted to the ISU-VDL were also tested using the ELISA kit.

IPMA: Confirmation of results from the ELISA was carried out by submitting the samples for the immunoperoxidase monolayer assay (IPMA) test to the Veterinary Diagnostic Lab of the University of Minnesota.

Results and Discussion

Three successive runs of samples of known samples provided by Boehringer Ingelheim Vetmedica, Inc. (BIVI) showed a 100% repeatability of results. Except for one positive sample which had a suspect status on the ISU-VDL ELISA and negative status on the BIVI-ELISA all other samples showed complete agreement when the testing was carried out at two different laboratories. It appeared that the IPMA was more sensitive than the ELISA as all known status samples were correctly detected as either positive or negative by this test (Table 1).

Examination of 308 serum samples from cases submitted to the ISU-VDL revealed that seven of eight herds were sero-positive for *L. intracellularis*. The within herd prevalence rates ranged from 10% to 100% (Table 2). However, it is noteworthy that sampling was not random as case submissions are usually for suspected cases.

An Immuno-fluorescence Assay (IFA) for *Lawsonia intracellularis* is currently under optimization at the Serology section of the ISU-VDL for confirmation of ELISA suspect cases.

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Initials Tests

Table1. Comparison of ISU-VDL ELISA with BIVI-ELISA and IPMA.

Sample	ISU-VDL ELISA	BIVI ELISA	UMN IPMA
Known negatives	20/20	20/20	20/20
Known Positives	19/20 (1 suspect)	19/20 (1 negative)	20/20

Sample Submission

Samples that were received in the Serology section before noontime will have results available on that same day. A fee of \$8.00 per sample is charged. Clients are requested to submit at least one ml of serum in double snap cap tubes. The link below will provide more information about the test and online submission:

<http://vetmed.iastate.edu/diagnostic-lab>

Table2. Prevalence of *Lawsonia intracellularis* from eight cases submitted at the ISU-VDL from various herds.

Case	Positives	Negatives	Suspects
1	5/50 (10%)	40/50 (80%)	5/50 (10%)
2	88/180 (49%)	70/180 (39%)	22/180 (12%)
3	10/10 (100%)	0/10 (0%)	0/10 (0%)
4	0/10 (0%)	10/10 (100%)	0/10 (0%)
5	15/30 (50%)	14/30 (47%)	1/30 (3%)
6	10/10 (100%)	0/10 (0%)	0/10 (0%)
7	10/10 (100%)	0/10 (0%)	0/10 (0%)
8	6/8 (75%)	1/8 (12.5%)	1/8 (12.5%)
TOTAL	144/308 (47%)	135/308 (44%)	29/308 (9%)