Short Communications

Longitudinal evaluation of three commercial diagnostic assays for *Lawsonia intracellularis* infection in pigs

A. Lebret, V. Auvigne, A. Morel Saives

ILEITIS is an intestinal disease of pigs commonly caused by *Lawsonia intracellularis*. Clinical signs range from bloody diarrhoea with sudden death to intermittent diarrhoea and retarded growth. Our study compared two serological and one antigen-based commercial diagnostic assay for the detection of *L intracellularis* infection in live animals: the bioScreen Ileitis Antibody ELISA (antibody ELISA), the Elanco IFAT IleiTest (immunofluorescent IgG detection) and the Elanco FIRSTtest (antigen ELISA).

The study was conducted in a 250-sow indoor farrow-to-finish farm known to be infected with *L intracellularis*. Piglets were weaned at 28 days of age and transferred to grower facilities at 12 weeks of age. Two batches of pigs weaned at a three-week interval in September 2010 were monitored from 5 to 23 (batch 2) or 26 (batch 1) weeks of age. Eleven pigs in each batch were randomly selected and individually identified. Every three weeks each of these pigs was bled and approximately 1 g of faeces was collected per rectum.

ELISA bioScreen tests were undertaken at the Laboratory Bio Chêne Vert (Chateaubourg, France); IFATs and FIRSTtests were performed at the Laboratory LDA22 (Ploufragan, France). Serum samples were analysed using the bioScreen Ileitis Antibody ELISA test, and absorbance values were converted into percentages of inhibition values (PI) as per the manufacturer's instructions (bioScreen, Münster, Germany). Qualitative ELISA results were determined using three different thresholds: PI 20 (indeterminate cut-off value recommended by the manufacturer), PI 30 (positive cut-off value recommended by the manufacturer) and PI 35 (cut-off value recommended by Jacobson and others 2011).

Sera were also analysed and interpreted using the Elanco IFAT IleiTest, as per the manufacturer's instructions (Elanco Animal Health, Indianapolis, Indiana, USA). Samples were considered test positive or negative based on the recommended values of the manufacturer when fluorescence to detect *L intracellularis* antibodies was compared with positive and negative standards in the kit.

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A. Lebret, DVM,
Porc.Spective – Chêne Vert Conseil,
Noyal-Pontivy 56920, France
V. Auvigne, DVM,
Ekipaj, 22 rue d'Assas, Angers 49000,
France
A. Morel Saives, DVM,
ELANCO Animal Health France,
24 boulevard Vital Bouhot,
Neuilly sur Seine 92521,
France

Email for correspondence:
a.lebret@chenevertconseil.com

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The FIRSTtest is a qualitative, single-use, ELISA-based colorimetric test for the detection of viable and non-viable *L intracellularis* in porcine faeces. The kit contains an anti-*Lawsonia* polyclonal antibody-peroxidase conjugate which is bound to magnetic beads. The polyclonal antibody is used in the separation phase to bind *L intracellularis* antigen, while the peroxidase is used during the detection phase to generate a quantifiable colour signal. A sample is considered positive if the colour intensity is stronger than that of the negative control (Kiachopoulos and Tice 2012).

Data analyses were undertaken on 145 of the 165 possible samples where the three tests had been performed. Results of the longitudinal study were summarised as the proportion of animals giving a positive result for the IFAT and antigen ELISA, or the proportion in various ranges of ELISA reactivity for the antibody ELISA. The degree of agreement between each pair of tests was determined by a κ test (VassarStats software, http://faculty.vassar.edu/lowry/kappa.html).

Seroconversion in the antibody ELISA occurred in all the pigs (Fig 1). No signal was detected in pigs aged 5–11 weeks (all PI<10) while 14–20-week-old pigs often yielded indeterminate (20≤PI<30) or negative values, the latter often in the range of PI 10–20. High positive (PI>70) cases were mainly detected among 17-week-old pigs. In older animals, positive results were most often within the range of PI 50–70. In the IFAT, one seropositive five-week-old pig was detected in batch 1, while other pigs did not seroconvert before 14–17 weeks of age. One pig remained IFAT negative throughout the study. The antigen ELISA indicated that shedding occurred at two time periods: in the weaner phase (at 5–8 weeks of age) and in the late grower phase (14–20 weeks of age), whereas, no shedding was detected in piglets over 20 weeks old.

According to a published interpretation of κ values (Landis and Koch 1977), the agreement between antibody ELISA and IFAT was found to be 'almost perfect'. The closest agreement was observed using the cut-off value of PI 30 as recommended by the manufacturer. Agreement between the antigen ELISA and the two other tests was 'slight' (Table 1).

Considering agreement with the IFAT, raising the antibody ELISA threshold to 35 as advocated by Jacobson and others (2011) had no advantage, as the best agreement was found when using the threshold value recommended by the manufacturer (PI 30). Pigs with an antibody ELISA result in the range of PI 10–30 were only found at 14–20 weeks of age, coinciding with the initial period of seroconversion in the herd. Thus, the low PI values at 14–20 weeks were considered to represent a specific serological signal, interpreted as 'undergoing seroconversion'.

Since the antibody ELISA and IFAT show 'almost perfect' agreement, it is pointless to apply these tests simultaneously. Our study did, however, reveal an advantage for the ELISA over the IFAT, notably in the semiquantitative nature of the results which can assist interpretation. For example, in batch 1 at 14 weeks of age, antibody ELISA results provided evidence of pigs undergoing seroconversion (10≤PI<30) in two pigs and seroconversion (PI≥30) in two others, when only one animal was positive in the IFAT. However, the IFAT was the only test to suggest the presence of residual maternally derived antibody in one five-week-old piglet, which is considered to be the age limit for detection of maternal antibodies to *L intracellularis* (Guedes and others 2002).

The lack of agreement between the antigen-based test and the serological tests can be attributed to the fact that these are measuring different aspects of *L intracellularis* infection. It also indicates that a combination of a serological test with the FIRSTtest may be more informative of the stage of infection. Thus, the FIRSTtest indicated shedding in the weaner phase (at five weeks), which could be attributed to sow-derived contamination, but was not associated with immediate seroconversion, as all eight- and 11-week-old pigs were seronegative. This may be due to an insufficient bacterial load to

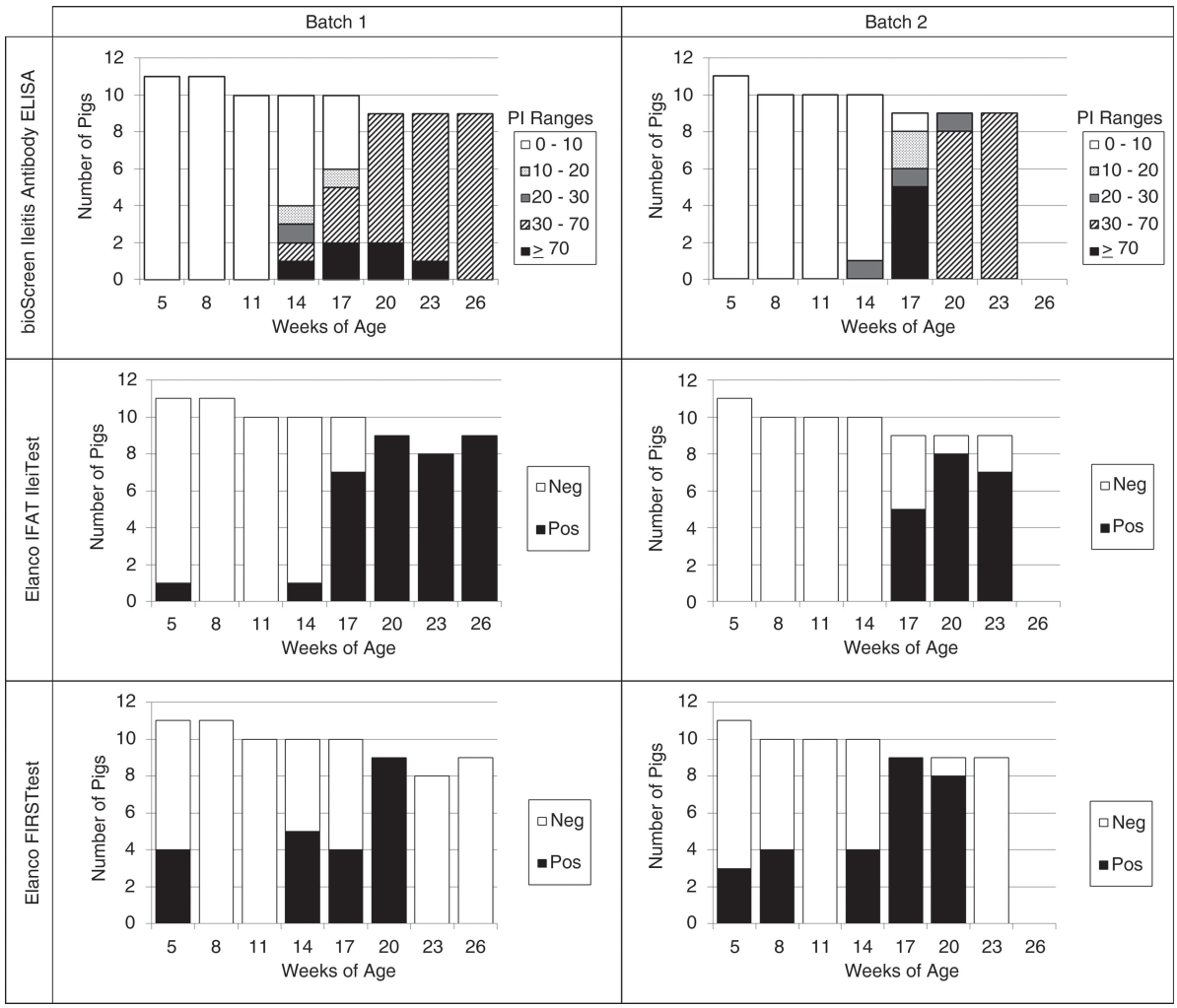

FIG 1: Longitudinal results in three diagnostic assays for *Lawsonia intracellularis* in 11 selected pigs from two separate batches, where each individual was sampled three-weekly between weaner and finisher age. (PI, percentage inhibition)

TABLE 1: Summary of agreements found between different assays for individual results

Assays compared	Agreement observed (%)	κ (95% CI)	Agreement quality*
ELISA cut-off 20 v IFAT	93	0.85 (0.77 to 0.94)	Almost perfect
ELISA cut-off 30 v IFAT	96	0.91 (0.84 to 0.98)	Almost perfect
ELISA cut-off 35 v IFAT	92	0.83 (0.74 to 0.93)	Almost perfect
IFAT v FIRSTtest	61	0.16 (0.00 to 0.32)	Slight
ELISA cut-off 30 v FIRSTtest	60	0.12 (0.00 to 0.30)	Slight

*Derived from Landis and Koch (1977)

induce seroconversion (Guedes and Gebhart 2003, Collins and Love 2007), or a lack of specificity in the antigen ELISA. Further studies on the specificity of this assay are required.

The repeatability of findings between the two batches of pigs suggests that reliable results may be obtainable through transverse sampling (herd profiling), where several batches of different ages are sampled concurrently. This has advantages in time and labour costs over longitudinal sampling procedures, where animals are repeatedly sampled at different time points. If the antibody ELISA is used, the age when a sampled group of pigs is undergoing seroconversion will be that when the entire range of PI values is present.

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Correction notice This article has been corrected since it was published Online First. In the title, 'swine' has changed to 'pigs', and headings throughout the article have been removed.

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