



## First evidence of *Lawsonia intracellularis* detection in air from commercial swine farms

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### ARTICLE INFO

#### Keywords:

Airborne  
Environmental sampling  
Ileitis  
*Lawsonia intracellularis*  
Porcine proliferative enteropathy  
Surveillance  
Swine

### ABSTRACT

*Lawsonia intracellularis* is the etiological agent of porcine proliferative enteropathy (PPE), a major enteric disease present in most swine herds worldwide. PPE expression is influenced by several factors, making continuous surveillance essential to minimize its impact. This study evaluated the feasibility and diagnostic performance of environmental sampling for the detection of *L. intracellularis* in commercial swine farms with subclinical and PPE infections. Three farms (A–C) were included: Farms A and B exhibited subclinical infection, while Farm C was affected by a PPE outbreak. Longitudinal sampling included serum, fecal swabs, air and surface samples from 10 to 11 to 17–18 weeks of age. Serology and quantitative PCR (qPCR) were used to monitor infection and quantify *L. intracellularis* loads. *L. intracellularis* infection was confirmed in all three farms using standard diagnostic methods. DNA of *L. intracellularis* was consistently detected in air and surface samples, with distinct temporal patterns across farms. In subclinical infected herds, early low-level detection in air and surface samples preceded widespread shedding, which was subsequently reflected in increased seroprevalence. In the PPE affected farm, high seropositivity and fecal shedding were observed during the outbreak, followed by a gradual decline. These dynamics were also mirrored by the quantity of *L. intracellularis* DNA detected both in air and surfaces over time. These findings demonstrate that environmental monitoring via air and surface sampling is might be a possible tool in the future to predict infection dynamic under both subclinical and clinical PPE conditions, providing a complementary method for the early detection and surveillance of *L. intracellularis*.

### 1. Introduction

*Lawsonia intracellularis* is the etiological agent of porcine proliferative enteropathy (PPE), one of the most significant enteric diseases affecting pigs during the grower–finisher phase (Vannucci et al., 2025). *L. intracellularis* infection is present in the vast majority of commercial swine herds worldwide (Arnold et al., 2019; Wang et al., 2024), producing a clinical spectrum that ranges from subclinical cases, in which reduced growth rate may be the only detectable sign, to overt disease characterized by diarrhea and wasting, or, in severe cases, intestinal hemorrhage (Arnold et al., 2019; Wang et al., 2024; Lawson and Gebhart, 2000; Jacobson et al., 2010). Due to its negative impact on average

daily gain, feed conversion rate and mortality, PPE exerts a considerable economic burden on the global swine industry.

PPE expression is influenced by factors such as host immune status, the presence of co-infections, and on-farm management conditions, which collectively complicate early detection and increases the risk of sudden, severe disease episodes (Mølbak et al., 2008; Johansen et al., 2013; Bronsvort et al., 2001; Leite et al., 2018; Jensen et al., 2005). Consequently, continuous monitoring of infection levels within herds, using both clinical surveillance and laboratory diagnostic tools, is essential to promptly identify shifts in pathogen prevalence or virulence that could precipitate a clinical outbreak (Jacobson et al., 2010). Implementing systematic surveillance programs not only supports

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<https://doi.org/10.1016/j.tvj.2025.106529>

Accepted 4 December 2025

Available online 6 December 2025

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timely intervention but also contributes to more effective long-term control strategies, minimizing economic impact and improving herd health.

Disease surveillance in swine production represents an economic and labor-intensive undertaking (Romagosa et al., 2012). These constraints highlight the need to develop and validate alternative surveillance strategies, such as pooled diagnostic testing and environmental sampling. In the latter, swine production systems are at the forefront of methodological innovation, with ongoing research directed toward optimizing sampling schemes and detection protocols (Otake et al., 2010; Alonso et al., 2014; Corzo et al., 2013; López-Lorenzo et al., 2022; Vilalta et al., 2019; Garrido-Mantilla et al., 2019; Hernandez-Garcia et al., 2017; Ramirez et al., 2012). Nevertheless, given the wide diversity of pathogens relevant to swine production, the diagnostic performance of these techniques may vary substantially depending on the target organism. In the specific case of *L. intracellularis*, to date, although positivity in pen floor feces has been demonstrated in different studies and even compared with oral fluid samples (Eddicks et al., 2025), no scientific studies have explored its detection in the farm environment samples; consequently, its practical applicability for routine monitoring under commercial production conditions remains undetermined.

For the aforementioned reasons, the objective of this study was to assess the feasibility and diagnostic performance of air and surface sampling methods for the detection of *Lawsonia intracellularis* DNA throughout the grower–finisher phase in commercial swine herds.

## 2. Material and methods

### 2.1. Study design

The present study was conducted on three commercial swine fattening farms (A–C), with the following management and structural characteristics: in an all-in/all out management system, pigs entered the facilities at approximately 9 weeks of age and were provided with *ad libitum* access to feed and water. The facilities featured a central alley flanked by pens on both sides, floors that were partially or fully slatted, and automated ventilation systems by lateral windows.

No vaccine against *L. intracellularis* was used on any of the farms.

Pigs on all three farms were unvaccinated against *Lawsonia intracellularis*. Pigs from Farms A (n pigs = 360) and B (n = 380), both derived from the same sow herd, showed no clinical signs consistent with this infection during all grower–finisher phase. Moreover, previous batches of pigs on these farms had also not exhibited clinical signs, and there was no history of PPE. Pigs from Farm C (n = 310) experienced a clinical outbreak of porcine proliferative enteropathy (PPE), characterized by dark blood in the feces, anemia, pale mucous membranes, and sudden deaths. This outbreak had already originated at the end of the weaning phase, when pigs were 7–8 weeks old, at which time the diagnosis of PPE was confirmed based on lesions and the detection of *L. intracellularis* by PCR, and persisted into the early grower–finisher phase.

Farms A and B were visited when pigs were 10, 12, 14, 16, and 18 weeks of age, whereas Farm C was visited at 11, 13, 15, and 17 weeks of age. During each visit, serum samples were collected from the jugular vein of 20 randomly selected pigs; six air samples (50 L/min for 30 min each one) were taken using the air sampler MD8 Airport (Sartorius AG, Göttingen, Germany) with sterile gelatine filters of 80 mm in diameter and a pore size of 3 µm (Sartorius Stedim Biotech GmbH, Göttingen, Germany), the air sampler was placed at approximately 1 m height in the central alley at 1/3 and 2/3 of the length of the building; and surface samples that were taken by swabbing from the following samples: the central alley, the pen railing, the pig hopper, the pen wall and the pen floor (the protocol for sampling each one was previously described (López-Lorenzo et al., 2022) and it is indicated in Table 1. Besides, in Farm C fecal swabs were also collected from the same randomly selected pigs used for serum sampling.

**Table 1**  
Surface sampling protocol.

Sample	Protocol of sampling and swabbing (with sterile cotton swabs of 11 mm diameter)
Central alley	One hundred steps were taken wearing polyethylene boot covers. Afterwards, both boot covers were swabbed in zigzag from the toe region to the heel.
Pen railing	One meter length of the pen railing was swabbed in zigzag (the same point in all visits).
Hopper	Eight different hoppers (the same ones in all visits) were selected and an area of 25 × 25 cm was swabbed in each one. The hoppers were located in the same pens used for sampling the pen wall and the pen floor.
Pen wall	Eight different pens (the same ones in all visits) were selected and an area of 25 × 25 cm, located at the height of the snout in the resting area, was swabbed. The same pens were used for sampling the hopper and the pen floor.
Pen floor	One hundred steps were taken wearing polyethylene boot covers. Afterwards, both boot covers were swabbed in the same way as for the central alley. The same pens were used for sampling the hopper and the pen wall.

### 2.2. Laboratory analysis

The tubes containing the blood samples were centrifuged during 10 min at 3800 × g and the serum was subsequently separated and kept at – 30 °C until processed. The serum samples were analyzed to detect the presence of antibodies against *L. intracellularis* using the commercial kit AsurDXTM *Lawsonia intracellularis* Antibody Test (BioStone South-lake, EEUU). A serum sample was considered seropositive to *L. intracellularis* when the sample/positive (S/P) ratio was ≥ 30 %.

The filters from air sampling were transferred to Stomacher bags and 5 ml of sterile phosphate- buffered saline with 0.05 % Tween 20 (PBST, all reagents supplied by Sigma- Aldrich, Missouri, United States) was added to each one to dissolve them. The obtained solution was homogenized for one minute and left to settle down for 15 min. After that, 1 ml of supernatant from each solution was transferred to a sterile Eppendorf tube and kept at – 30 °C until the DNA extraction was performed.

Surface samples were processed by adding 5 ml of PBST to each tube containing the swab. They were vortexed for one minute and subsequently left to settle down for 15 min. After that, 1 ml of supernatant from each sample was placed in a sterile Eppendorf tube and kept at – 30 °C until the DNA extraction was performed.

Fecal swabs were placed in sterile Eppendorf tubes, and the swab stick was cut to fit inside the tube. Subsequently, 1 ml of sterile PBST was added, and the samples were vortexed for one minute. Finally, the tubes were stored at – 30 °C until DNA extraction was performed.

DNA extraction was performed on the frozen Eppendorf tubes containing air samples, surface samples, and individual fecal swabs. A commercial DNA extraction kit (High Pure PCR Template Preparation Kit, Roche Diagnostics GmbH, Mannheim, Germany) was used according to the manufacturer's instructions, with 200 µl of each sample used as starting material and the extracted DNA eluted in 100 µl of elution buffer. An exogenous internal control (EXOone IPTC, EXOPOL S.L., Zaragoza, Spain) was added to each environmental sample to detect potential qPCR inhibition.

Quantitative PCR (qPCR) analysis was performed using a commercial kit (EXOone *Lawsonia intracellularis* oneMIX, EXOPOL S. L., Zaragoza, Spain), following the manufacturer's instructions. qPCR positive and negative controls were supplied by the manufacturer and were used in each run. A sample was considered positive to *L. intracellularis* when Cq ≤ 40 for the FAM detection channel. In addition, the positive control was used to calculate the standard curve by preparing serial ten-fold dilutions (5 × 10<sup>5</sup>–5 × 10<sup>1</sup> copies/µl). All qPCR reactions were run on an QuantStudio™ 5 Real-Time PCR System thermocycler (Thermo Fisher Scientific, Waltham, MA, USA).

The amount of *L. intracellularis* DNA quantified for each sample was

expressed as number of copies/m<sup>3</sup> of air and number of copies/swab for air and surface samples, respectively.

### 3. Results

#### 3.1. Standard curve

The number of *L. intracellularis* DNA copies was calculated using the standard curve obtained for this purpose ( $y = 40.256 - 3.411x$ );  $R^2 = 0.997$ ; efficiency = 96.432.

#### 3.2. Monitoring of *L. intracellularis* in reference samples (serum and fecal swabs)

A positive serological result for *L. intracellularis* was found in all three evaluated farms in at least one sampling. Farm A maintained a relatively stable proportion of seropositive pigs throughout the study, with values ranging from 5 % at 12 and 16 weeks of age to 15 % at 14 weeks of age. Farm B, which also presented a subclinical infection, exhibited a different serological pattern; the proportion of seropositive pigs was 0 % at 10 and 12 weeks of age, increasing to 75 % by the end of the following period. In contrast, Farm C, where pigs were affected by a clinical outbreak of PPE, started with higher seropositivity values (85 % in the first two visits) that subsequently decreased to 60 % at 17 weeks of age. The evolution of the within herd seroprevalence on each farm is presented in Fig. 1.

Regarding fecal swabs, this monitoring was only performed on Farm C. Similar to the antibody results, the highest percentage of positive pigs was observed during the first visit (65 %), followed by a gradual decrease (40 % at 13 weeks of age, 10 % at 15 weeks of age) until the last visit (0 %). However, despite this overall decline, the amount of *L. intracellularis* excretion displayed a different pattern, with an increase observed at 15 weeks of age (Fig. 1.C).

#### 3.3. Monitoring of *L. intracellularis* through air samples

The results for air samples are presented in Table 2. *L. intracellularis* was detected in air samples from all three farms, each one showing a different temporal pattern. In Farm A, detection in air samples was not observed until 16 weeks of age, when one sample tested positive (very low levels); in the subsequent visit, 5 of 6 air samples were positive, with more *L. intracellularis* DNA copies than in the previous visit (Fig. 1.A). In Farm B, the first detection occurred at 12 weeks of age, with one low positive sample; in subsequent visits, all air samples tested positive, with a number of *L. intracellularis* DNA copies comparable to those observed in the PPE-affected farm (Fig. 1.B and 1.C). In Farm C, all air samples tested positive throughout the study, with a peak detection in the first sampling and a progressive decrease in *L. intracellularis* quantity over time (Fig. 1.C).

#### 3.4. Monitoring of *L. intracellularis* through surface samples

The results for surface samples are presented in Table 3. Similar to the air samples, *L. intracellularis* DNA was detected on surfaces in all three farms, each exhibiting a distinct pattern. In Farm A, detection first occurred at 14 weeks of age, when DNA was detected on the pen floor. In the following visit, the pen floor remained the only positive site, whereas in the final visit all surface samples, except the pen railing, tested positive, with higher amount of *L. intracellularis* DNA (Fig. 1.A). In Farm B, the first detection occurred at 12 weeks of age, and in subsequent visits all surface samples tested positive, reaching a peak in the final sampling. Notably, pen floor samples consistently showed high *L. intracellularis* amount, ranging from  $5.39 \times 10^6$  to  $1.94 \times 10^7$  DNA copies per swab, except at the first detection. Finally, in Farm C, all surface samples tested positive at all time points, except for the feeder in the last visit, which was negative. In this farm, the amount of *L. intracellularis* in surface

samples decreased over time (Fig. 1.C).

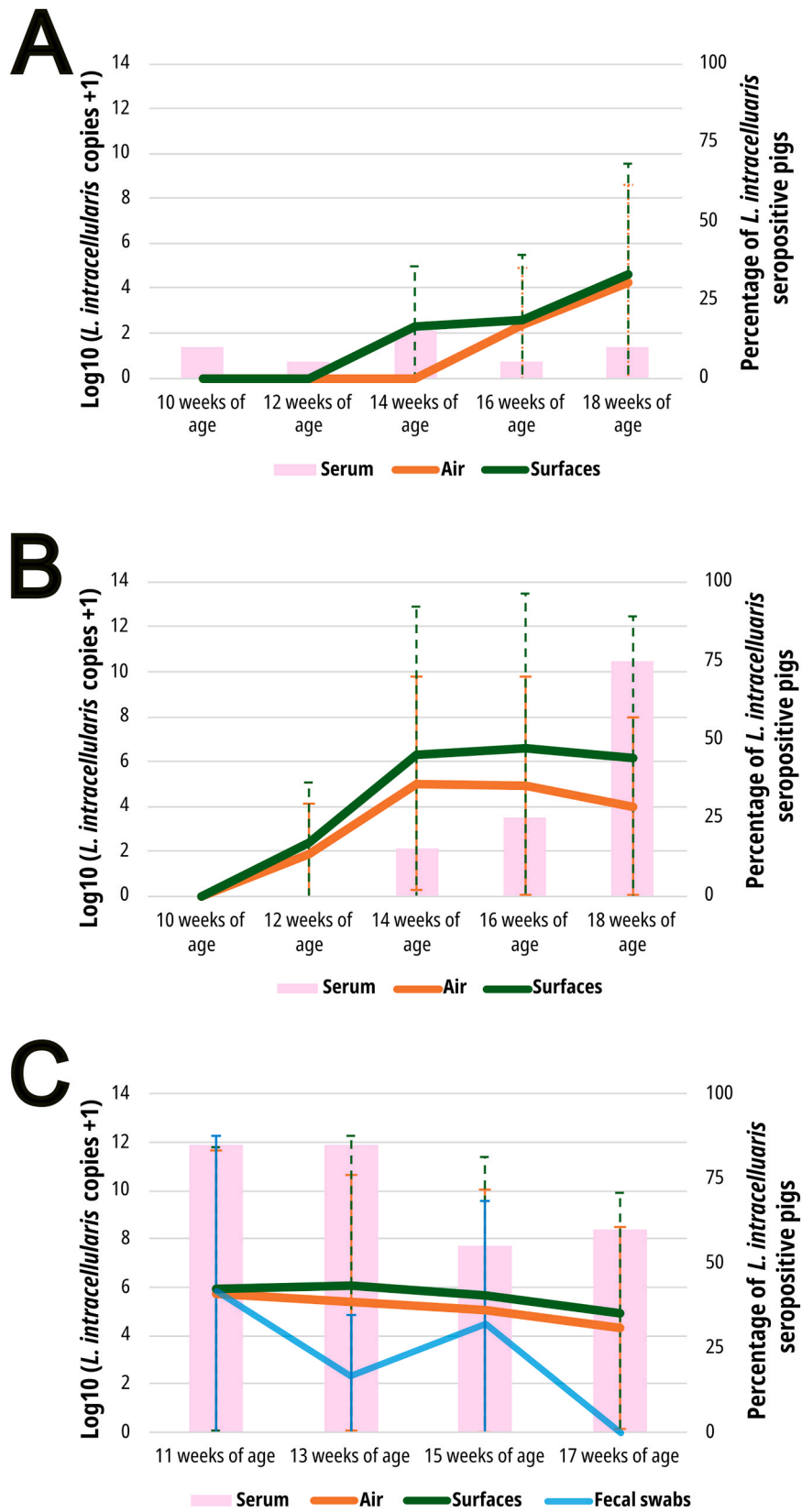
### 4. Discussion

This study demonstrates that *Lawsonia intracellularis* DNA can be consistently detected in air and surface samples under commercial production conditions. To our knowledge, this is the first work evaluating the applicability of air sampling approach for the monitoring of *L. intracellularis* in swine herds. These results highlight that different infection patterns can be manifested in the dynamics of environmental contamination and, emphasize the potential of environmental monitoring as a complementary strategy for the early detection and routine surveillance of porcine proliferative enteropathy in pig production systems.

A noteworthy finding of the present study was that, despite originating from the same source herd and showing no clinical signs of PPE, pigs housed in Farms A and B exhibited markedly divergent infection dynamics. In both herds, the proportion of *L. intracellularis* seropositive pigs was low during the first sampling; however, the temporal progression of infection differed substantially between sites. This suggests that management related factors during the grower-finisher phase such as cleaning and disinfection, pig density... can modulate the infection pressure. One of these factors may be the presence of rodents. In Farm B numerous indicators of rodent activity were documented. Rodents are recognized as potential reservoirs and mechanical vectors for *L. intracellularis* transmission; moreover, direct transmission between rodents and pigs has also been demonstrated (Friedman et al., 2008; Pearson et al., 2016; Gabardo et al., 2017). The latter may partially explain the pronounced increase in seropositive pigs observed over the course of the study. Another plausible contributing factor is that the previous production batch housed in this facility had experienced a PPE outbreak, and the routine cleaning and disinfection procedures performed prior to pig placement may have been insufficient for complete pathogen removal (Gómez-García et al., 2022). However, this possibility seems less likely, as initial air and PCR samples were negative, indicating that even if viable bacteria were present, their quantity would have been below the detection limit. Collecting fecal swabs in Farms A and B, as was performed in Farm C, might have provided additional insight to further elucidate these hypotheses; however, it should be noted that these farms were not originally sampling to investigate PPE dynamics, but rather other swine infections. Thus, these findings highlight an important aspect to be addressed and systematically evaluated in future studies.

Fecal swabbing in Farm C provided more specific insights into *L. intracellularis* dynamics. Notably, at the time of the first sampling, the peak of bacterial shedding was observed, indicating that transmission had already occurred in most of the pigs during the nursery phase. Although such early infection is uncommon, it has been previously reported by other authors (Arnold et al., 2019; Stege et al., 2004; Brandt et al., 2010). Like previous descriptions of *L. intracellularis* shedding dynamics, fecal shedding in the present study persisted for slightly over one month. However, our results revealed a decline in the proportion of seropositive pigs during the last two samplings, which contrasts with previous reports where the highest seropositivity was detected at the end of the finishing phase (Arnold et al., 2019; Jensen et al., 2005; Stege et al., 2004). This discrepancy could be mainly attributed to timing between the infection and the sampling, although we cannot totally discard differences in the assays used to measure the immune response (IFAT vs. ELISA) or to the specific commercial ELISA employed. In our case, an indirect ELISA that measures only IgG was used, whereas the more commonly employed commercial ELISA detects total immunoglobulins (Campillo et al., 2021). This methodological difference may account for the lower seropositivity observed at the end of our study.

Regarding air and surface sampling, this is the first study demonstrating the detection of *L. intracellularis* DNA in the airborne environment and surfaces from swine facilities. Considered that *L. intracellularis*



**Fig. 1.** Monitoring of *L. intracellularis* in three farms (A–C) using different samples types. Bars represent the percentage of seropositive pigs against *L. intracellularis*. Lines represent temporal dynamics of *L. intracellularis* DNA copies by qPCR ( $\log_{10}$  mean  $\pm$  standard deviation) in air (orange line; copies/ $m^3$  air), surfaces (green line; copies/swab) or fecal swabs (blue line; copies/swab) in each farm. Negative results were assigned a value of 1 DNA copy for graphical purposes. A. Farm A: PPE unaffected farm. B. Farm B: PPE unaffected farm. C. Farm C: PPE affected farm.

**Table 2**  
Number of *L. intracellularis* DNA copies per m<sup>3</sup> of air in each air sample.

FARM	SAMPLING POINT	Weeks of age									
		10	11	12	13	14	15	16	17	18	
A	1	-	-	-	-	-	-	-	-	-	6.26 × 10 <sup>0</sup>
	2	-	-	-	-	-	-	-	-	-	3.00 × 10 <sup>3</sup>
	3	-	-	-	-	-	-	4.85 × 10 <sup>2</sup>	-	-	8.26 × 10 <sup>3</sup>
	4	-	-	-	-	-	-	-	-	-	1.64 × 10 <sup>3</sup>
	5	-	-	-	-	-	-	-	-	-	2.60 × 10 <sup>4</sup>
	6	-	-	-	-	-	-	-	-	-	-
B	1	-	-	-	-	7.17 × 10 <sup>4</sup>	-	8.26 × 10 <sup>3</sup>	-	-	6.80 × 10 <sup>2</sup>
	2	-	-	-	-	4.78 × 10 <sup>4</sup>	-	6.70 × 10 <sup>4</sup>	-	-	1.62 × 10 <sup>4</sup>
	3	-	-	-	-	7.67 × 10 <sup>4</sup>	-	4.18 × 10 <sup>4</sup>	-	-	2.60 × 10 <sup>4</sup>
	4	-	-	-	-	1.97 × 10 <sup>5</sup>	-	8.78 × 10 <sup>4</sup>	-	-	5.90 × 10 <sup>3</sup>
	5	-	-	-	-	1.00 × 10 <sup>5</sup>	-	8.20 × 10 <sup>4</sup>	-	-	4.50 × 10 <sup>3</sup>
	6	-	-	5.19 × 10 <sup>2</sup>	-	1.23 × 10 <sup>5</sup>	-	2.11 × 10 <sup>5</sup>	-	-	8.26 × 10 <sup>3</sup>
C	1	-	1.72 × 10 <sup>5</sup>	-	4.15 × 10 <sup>5</sup>	-	2.96 × 10 <sup>5</sup>	-	3.90 × 10 <sup>4</sup>	-	-
	2	-	2.24 × 10 <sup>6</sup>	-	2.42 × 10 <sup>5</sup>	-	1.61 × 10 <sup>5</sup>	-	2.43 × 10 <sup>4</sup>	-	-
	3	-	1.61 × 10 <sup>5</sup>	-	5.43 × 10 <sup>5</sup>	-	1.00 × 10 <sup>5</sup>	-	5.51 × 10 <sup>3</sup>	-	-
	4	-	1.07 × 10 <sup>5</sup>	-	1.00 × 10 <sup>5</sup>	-	3.41 × 10 <sup>4</sup>	-	3.93 × 10 <sup>3</sup>	-	-
	5	-	1.07 × 10 <sup>5</sup>	-	9.39 × 10 <sup>4</sup>	-	3.65 × 10 <sup>4</sup>	-	1.16 × 10 <sup>4</sup>	-	-
	6	-	2.96 × 10 <sup>5</sup>	-	4.47 × 10 <sup>4</sup>	-	3.19 × 10 <sup>4</sup>	-	3.90 × 10 <sup>4</sup>	-	-

- Indicates a negative result to *L. intracellularis* DNA.

**Table 3**  
Number of *L. intracellularis* DNA copies per swab in each surface sample.

FARM	SAMPLING POINT	Weeks of age									
		10	11	12	13	14	15	16	17	18	
A	Central alley	-	-	-	-	-	-	-	-	-	6.80 × 10 <sup>2</sup>
	Pen railing	-	-	-	-	-	-	-	-	-	-
	Hopper	-	-	-	-	-	-	-	-	-	2.45 × 10 <sup>3</sup>
	Pen wall	-	-	-	-	-	-	-	-	-	3.93 × 10 <sup>3</sup>
	Pen floor	-	-	-	-	1.02 × 10 <sup>3</sup>	-	1.86 × 10 <sup>3</sup>	-	-	1.90 × 10 <sup>5</sup>
B	Central alley	-	-	-	-	1.01 × 10 <sup>4</sup>	-	1.08 × 10 <sup>5</sup>	-	-	2.77 × 10 <sup>5</sup>
	Pen railing	-	-	-	-	2.96 × 10 <sup>5</sup>	-	1.41 × 10 <sup>5</sup>	-	-	3.39 × 10 <sup>5</sup>
	Hopper	-	-	-	-	1.61 × 10 <sup>5</sup>	-	2.13 × 10 <sup>4</sup>	-	-	9.97 × 10 <sup>5</sup>
	Pen wall	-	-	-	-	2.77 × 10 <sup>5</sup>	-	5.08 × 10 <sup>5</sup>	-	-	1.72 × 10 <sup>5</sup>
	Pen floor	-	-	1.17 × 10 <sup>3</sup>	-	9.25 × 10 <sup>6</sup>	-	1.94 × 10 <sup>7</sup>	-	-	5.39 × 10 <sup>6</sup>
C	Central alley	-	1.96 × 10 <sup>6</sup>	-	2.75 × 10 <sup>6</sup>	-	1.61 × 10 <sup>5</sup>	-	2.11 × 10 <sup>5</sup>	-	-
	Pen railing	-	33.65 × 10 <sup>4</sup>	-	2.24 × 10 <sup>6</sup>	-	1.24 × 10 <sup>4</sup>	-	5.15 × 10 <sup>3</sup>	-	-
	Hopper	-	3.88 × 10 <sup>5</sup>	-	1.97 × 10 <sup>5</sup>	-	8.78 × 10 <sup>4</sup>	-	-	-	-
	Pen wall	-	5.81 × 10 <sup>5</sup>	-	1.97 × 10 <sup>5</sup>	-	1.31 × 10 <sup>6</sup>	-	1.24 × 10 <sup>4</sup>	-	-
	Pen floor	-	1.40 × 10 <sup>6</sup>	-	3.91 × 10 <sup>4</sup>	-	6.65 × 10 <sup>5</sup>	-	1.51 × 10 <sup>5</sup>	-	-

- Indicates a negative result to *L. intracellularis* DNA.

is an obligate intracellular bacterium that is excreted via feces, it can directly or indirectly contaminate different farm surfaces (Filippitzi et al., 2018). Our results are coherent with this information, as the pen floor consistently harbored the highest bacterial loads, whereas the amount of *L. intracellularis* DNA detected on the rest of surfaces varies depending on the sampling. For airborne detection, the most plausible mechanism involves the drying swine feces followed by the resuspension of contaminated particles into the barn dust, thereby facilitating aerogenous dissemination. This hypothesis has also been suggested for other digestive pathogens which airborne detection has been successful, such as Porcine Epidemic Diarrhea Virus (Alonso et al., 2014).

We detected positive results both in the air and surface samples across the three farms, with consistent detection since the first sampling in the clinically affected herd. In contrast, in the subclinically infected herds, a single air sample and a pen floor sample tested positive at low bacterial loads prior to the presumed onset of widespread shedding. Specifically, air samples were positive at 16 weeks of age on Farm A and 12 weeks on Farm B, while surface samples were positive at 14 weeks on Farm A and 12 weeks on Farm B. After this point, all subsequent air samples tested positive. We cannot confirm this increase in shedding in Farm A due to the finishing of the monitoring; however, results from Farm B further support this interpretation: the first detection in both air and pen floor samples preceded the marked rise in seroprevalence by

approximately two to four weeks, consistent with the expected interval between the onset of *L. intracellularis* shedding and the development of a measurable humoral immune response (Jensen et al., 2005; Stege et al., 2004). Thus, the results of this study suggest that both the air sampling as well as surface sampling methodologies employed could serve as a valuable tool for the early detection of *L. intracellularis* shedding in swine herds, enabling the adaptation of control strategies and even vaccination programs aimed at minimizing PPE prevalence and its economic impact.

Environmental monitoring in the farms with the highest proportion of positive samples (Farms B and C) also revealed that the quantity of *L. intracellularis* DNA was comparable in both herds (around 10<sup>5</sup> and 10<sup>6</sup> *L. intracellularis* DNA copies for air and surface samples), despite their markedly different clinical status. In fact, Farm B, despite being subclinically infected, consistently exhibited higher levels of *L. intracellularis* DNA on pen floors than Farm C. This finding contrasts with previous information, which indicates that pigs clinically affected by PPE are typically the ones excreting the highest amounts of *L. intracellularis* (Pedersen et al., 2012; Collins and Barchia, 2014). This observation underscores that, although environmental sampling of *L. intracellularis* appears highly promising, further research is required to optimize its implementation. For instance, in Farms B and C, the amount of *L. intracellularis* DNA detected in air samples gradually declined after the observed peak, with a more pronounced reduction as pigs aged. This

pattern likely reflects the influence of barn ventilation, since as pigs grow, farm ventilation is higher and entails a more rapid reduction of bacterial loads in airborne dust (Corzo et al., 2013). Conversely, bacterial levels on surfaces depend primarily on the magnitude of fecal shedding, bacterial persistence on surfaces and pig activity, which explains the comparatively less pronounced decrease observed in surface samples. Consequently, further research is warranted to establish correlations between the quantity of *L. intracellularis* detected in air and/or surface samples and the bacterial shedding by pigs, as well as to evaluate the applicability of such environmental monitoring approaches in control programs and their cost-effectiveness relative to conventional surveillance strategies.

Finally, an aspect that was intentionally excluded from the design of this study should be mentioned. Vaccination against *L. intracellularis* is widely implemented in swine production systems across many countries to reduce the incidence and severity of porcine proliferative enteropathy (PPE), as well as associated production losses (Won et al., 2022). Environmental monitoring may represent a highly valuable tool for assessing the efficacy of vaccination programs against *L. intracellularis*, as it enables the observation of temporal reductions in bacterial load within production facilities. In the case of inactivated bacterin-based vaccines, the proposed methodology could be directly applied since no shedding of *L. intracellularis* occurs (Jacobs et al., 2020). However, when using live attenuated vaccines, potential interference between the detection of vaccine-derived strains and field bacteria may arise, specifically in the first days after vaccination (Leite et al., 2021). Therefore, further research is warranted to address this limitation and to refine environmental detection approaches for *L. intracellularis* in the context of vaccination.

## 5. Conclusion

This study provides the first evidence that air and surface sampling can be employed to detect *Lawsonia intracellularis* in commercial swine farms, both under subclinical conditions and during clinical outbreaks of porcine proliferative enteropathy. The methodologies applied enabled the early detection of infection, which was subsequently followed by an increase in seroprevalence approximately two to four weeks later. Importantly, the results also demonstrate that herds with subclinical infections can contaminate the farm environment with *L. intracellularis* at levels comparable to those observed in herds clinically affected by PPE.

## CRedit authorship contribution statement

**Gonzalo López-Lorenzo:** Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Ana Carvajal:** Writing – original draft, Methodology, Conceptualization. **Alfredo Benito:** Writing – original draft, Methodology, Conceptualization. **Alberto Prieto:** Writing – review & editing, Methodology. **Mireya Melero:** Writing – review & editing, Methodology. **Gonzalo Fernández:** Writing – review & editing, Funding acquisition. **José Manuel Díaz-Cao:** Writing – review & editing, Supervision.

## Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use generative artificial intelligence (AI) and AI-assisted technologies in any part of the study development and in the paper redaction.

## Institutional Review Board Statement

The procedures which involved live animals (blood sampling) were approved by the bioethics committee of the University of Santiago de Compostela (project identification code: 2019-CE194).

## Funding

This research was supported by the Programme for Consolidating and Structuring Competitive Research Groups (GRC2019/04; Xunta de Galicia, Spain) and by a postdoctoral grant to G. López-Lorenzo (Campus de especialización Campus Terra, Universidade de Santiago de Compostela).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

We are very grateful for the collaboration and willingness of the farmers during the sampling process. At same time, we want to thank María del Mar Lara for her technical assistance.

## Data Availability Statement

The data are available from the corresponding author upon reasonable request.

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