

Detection of *Lawsonia intracellularis* by oral fluids and fecal samples in Canadian swine

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Summary

Objectives: The study objectives were to 1) describe the proportion of *Lawsonia intracellularis*-positive samples in unvaccinated and vaccinated Canadian swine herds during the mid- and late-finishing phases; 2) compare the probability of detecting *L intracellularis* by quantitative polymerase chain reaction using fecal samples (FS) and oral fluids (OF); and 3) investigate risk factors of *L intracellularis* detection using FS and OF.

Material and methods: Site demographics and vaccination protocols were obtained from 40 Canadian swine sites via questionnaire. Three OF and 3 FS were collected per site once during the

mid-finisher (15-17 wk of age) and once during the late-finisher (20-22 wk of age) production stages.

Results: Half of all investigated production sites were positive for *L intracellularis*. A 2-fold increase in *L intracellularis* detection rate was observed for OF compared to FS (odds ratio = 2.36; 95% CI, 1.24-4.49; $P = .009$). The presence of porcine circovirus type 2 (PCV2) had a 5-fold increased risk of *L intracellularis* positivity compared to sites without PCV2 (incidence rate ratio [IRR] = 4.99; 95% CI, 1.29-20.23; $P = .02$). A higher positive rate was found for sites with *L intracellularis* outbreaks within the last 2 years (IRR = 3.08; 95% CI, 1.51-6.37; $P = .002$).

Implications: This study presents evidence that OF may have a higher detection rate compared to FS for *L intracellularis*. Herds with PCV2 or exposure to recent *L intracellularis* outbreaks may be at increased risk of harboring *L intracellularis* and warrant additional investigation.

Keywords: swine, proliferative enteropathy, ileitis, vaccination, detection

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Resumen - Detección de *Lawsonia intracellularis* por fluidos orales y muestras fecales en cerdos canadienses

Objetivos: Los objetivos del estudio fueron 1) describir la proporción de muestras positivas para *Lawsonia intracellularis* en piaras porcinas canadienses vacunadas y no vacunadas durante las fases de engorde medio y tardío; 2) comparar la probabilidad de detectar *L intracellularis* mediante la reacción en cadena de la polimerasa cuantitativa utilizando muestras fecales (MF) y fluidos orales (FO); y 3) investigar los factores de riesgo de la detección de *L intracellularis* mediante MF y FO.

Material y métodos: Mediante un cuestionario se obtuvieron datos demográficos y protocolos de vacunación de 40

sitios porcinos canadienses. Se recolectaron tres FO y 3 MF por sitio, una vez durante las etapas de producción de engorde medio (15-17 semanas de edad), y una vez durante las etapas de producción de engorde tardío (20-22 semanas de edad).

Resultados: La mitad de todos los sitios de producción investigados fueron positivos a *L intracellularis*. Se observó un aumento de 2 veces en la tasa de detección de *L intracellularis* para la FO en comparación con la MF (coeficiente de correlación = 2.36; IC 95%, 1.24-4.49; $P = .009$). La presencia de circovirus porcino tipo 2 (PCV2) tuvo un riesgo 5 veces mayor de positividad para *L intracellularis* en comparación con los sitios sin PCV2 (cociente de tasa de incidencia [CTI] = 4.99; IC 95%,

1.29-20.23; $P = .02$). Se encontró una tasa positiva más alta en los sitios con brotes de *L intracellularis* en los últimos 2 años (CTI = 3.08; IC 95%, 1.51-6.37; $P = .002$).

Implicaciones: Este estudio presenta evidencia de que la FO puede tener una tasa de detección más alta en comparación con la MF para *L intracellularis*. Las piaras con PCV2 o exposición a brotes recientes de *L intracellularis* pueden tener un mayor riesgo de albergar *L intracellularis* y justificar una investigación adicional.

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Résumé - Détection de *Lawsonia intracellularis* par échantillonnage de fluides oraux et échantillons fécaux chez des porcs canadiens

Objectifs: Les objectifs de l'étude étaient de 1) décrire la proportion d'échantillons positifs à *Lawsonia intracellularis* dans des troupeaux de porcs canadiens vaccinés et non-vaccinés au milieu et à la fin de la phase de finition; 2) comparer la probabilité de détecter *L intracellularis* par réaction d'amplification en chaîne par la polymérase quantitative en utilisant des échantillons fécaux (FS) et des fluides oraux (OF); et 3) étudier les facteurs de risques de la détection de *L intracellularis* en utilisant des FS et des OF.

Matériels et méthodes: Les données démographiques des sites et les protocoles de vaccination ont été obtenus de 40 sites porcins via un questionnaire. Trois OF et 3 FS ont été prélevés par site, une fois vers le milieu de la période de finition (15-17 semaines d'âge) et une fois vers la fin de la période de finition (20-22 semaines d'âge).

Résultats: La moitié des sites de production étudiés étaient positifs pour la présence de *L intracellularis*. Une augmentation du double dans les taux de détection était observée pour OF comparativement à FS (rapport de cotes = 2.36; IC 95%, 1.24-4.49; $P = .009$). La présence de circovirus porcine type 2 (PCV2) multipliait par 5 l'augmentation du risque

de positivité à *L intracellularis* comparativement aux sites sans PCV2 (rapport du taux d'incidence [IRR] = 4.99; IC 95%, 1.29-20.23; $P = .02$). Un taux de positivité plus élevé a été trouvé pour les sites avec des poussées de cas de *L intracellularis* au cours des 2 dernières années (IRR = 3.08; IC 95%, 1.51-6.37; $P = .002$).

Implications: Cette étude présente des preuves que les OF pourraient avoir un taux de détection plus élevé comparativement au FS pour *L intracellularis*. Les troupeaux avec PCV2 ou des poussées de cas récentes de *L intracellularis* pourraient être plus à risque d'être positifs pour *L intracellularis* et le tout mérite des études additionnelles.

Porcine proliferative enteropathy, or ileitis, associated with the gram-negative obligate intracellular bacterium *Lawsonia intracellularis* remains a challenge for swine producers globally.^{1,2} The 2012 National Animal Health Monitoring System reported that 28.7% of US growing-finishing swine production sites had confirmed cases of ileitis.³ Ileitis is characterized by the thickening of the ileum mucosa with proliferated crypt epithelial cells, resulting in diarrhea, intestinal hemorrhaging, and weight loss.⁴ Disease severity varies, with increased mortality mainly seen in acute cases, while chronic and subclinical cases are mainly associated in high morbidity and poor growth performance.⁵ Although pigs often recover without intervention within a few weeks,⁴ the shedding and transmission of *L intracellularis* between infected and susceptible animals via feces are likely, causing additional costs due to hindered feed conversion and extra care from the producer.^{6,7} In addition, the cost of preventive management ranges from \$0.18 to \$1.00 per pig depending on vaccination strategy.⁸

The prevalence of *L intracellularis* in US and Canadian swine herds has been previously reported to be 75.0% to 96.0% and 16.7% to 100%, respectively.^{1,9,10} However, *L intracellularis* prevalence may differ significantly among geographical regions, production sites, and production phases within sites. For instance, within-herd prevalence variability has been reported to be up to 90.0% for sows, 11.0% to 92.0% for growing pigs, and 16.7% to 100% for finishing pigs.^{9,11,12} Thus, it has been suggested that approximately one-third of all grower and finisher pigs will be subjected to *L intracellularis* infection during their lifespan.¹⁰

Additionally, prevalence estimates may be influenced by local intervention approaches, sampling techniques, diagnostic tools used, and the sampling time post infection.¹³

Because of the difficulty in culturing *L intracellularis*, diagnosis has been widely accomplished by detecting *L intracellularis* DNA in fecal and intestinal tissue samples using polymerase chain reactions (PCR).¹⁴ However, quantifying *L intracellularis* DNA using fecal samples (FS) may yield inconsistent results with varied diagnostic performance due to differences in sample quality, herd prevalence, subclinical or clinical infection, the occurrence of lesions, and the number of samples analyzed.¹⁵

Alternatively, swine oral fluids (OF) have been successfully used as a diagnostic sample type for the detection of various swine pathogens (eg, porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus, and pseudorabies virus).^{16,17} Oral fluid collection reduces sample collection-associated animal stress and personnel labor cost and time. More recently, OF has also been used for *L intracellularis* antibody detection with a reported 100% specificity and 84.6% to 88.5% sensitivity for immunoglobulin A and immunoglobulin G, respectively, when compared to serum samples using an immunoperoxidase monolayer assay.¹⁸

The use of live attenuated oral and intramuscular inactivated vaccines against *L intracellularis* is one of the prevention tools available for swine veterinarians and producers. Even though their use has shown a reduction in intestinal lesion manifestation and mortality, the data is still controversial about reducing

fecal shedding of *L intracellularis*.¹⁹⁻²¹ Protective effects have also been reported to be dependent on the vaccine dose by showing dose-dependent increases in humoral and cell-mediated immunities for the live-attenuated ileitis vaccine, Enterisol.¹⁹

To date, there is limited knowledge on the association between *L intracellularis* vaccination, sample type, and grower pig production phase and *L intracellularis* detection using quantitative PCR (qPCR). Additionally, investigation on how vaccination protocols and other farm-level risk factors (eg, detection of other pathogens, historical detection and clinical observation of *L intracellularis* cases, and historical use of *L intracellularis* vaccine) may be associated with *L intracellularis* detection in herds has not been fully reported in the literature. Thus, the objectives of this study were to 1) describe the proportion of *L intracellularis*-positive samples in unvaccinated and vaccinated Canadian swine herds during the mid- and late-finishing phases; 2) compare the probability of detecting *L intracellularis* by qPCR using FS and OF; and 3) investigate risk factors of *L intracellularis* detection using FS and OF.

Animal care and use

Animal ethics review and approval were not required for the current study as all samples and data were collected by the herd veterinarians as part of their routine professional duties and existing veterinarian-client-patient relationship. All animals were housed and cared for under commercial swine conditions according to the Canadian National Farm Animal Care Council's Code of Practice for the care and handling of farm animals.

Materials and methods

A prospective cohort study design was implemented during June to October 2021 by enrolling 40 wean-to-finish swine production sites in the Canadian provinces of Ontario (ON = 18), Manitoba (MB = 20), and Quebec (QC = 2). The mean (SD) herd size was 3140 (2566) and pigs were conveniently enrolled through clients of 2 veterinary clinics in ON and MB. Recruitment was conducted based on veterinarian communication with clients through their professional network. Half of the enrolled sites (ON = 10; MB = 10) were actively vaccinated with an *L intracellularis* vaccine (Porcilis Ileitis, Merck Animal Health) before and during the study, whereas the remaining 20 sites (ON = 8; MB = 10, QC = 2) were unvaccinated.

Throughout the study period, each site was visited twice, once during the mid-finisher (15-17 weeks of age) phase and once during the late-finisher (20-22 weeks of age) phase. During these visits, 3 pens were conveniently selected at each site by the herd veterinarian, and 1 OF and 1 FS were collected from each pen per visit. The location of each sampled pen was spatially fixed between the 2 sampling events, ie, the same pens were sampled for the mid-finisher and late-finisher phases. Thus, 12 samples were collected per site ([1 OF + 1 FS] × 3 pens × 2 visits), culminating in a total of 480 samples for the study. Each sampling method included samples obtained from multiple individuals. Multiple fresh fecal samples were collected from the floor of pig pens and conveniently selected by the herd veterinarian aiming for a representative sample. Oral fluid samples were obtained from cotton ropes attached to each pen for 20 to 30 minutes on each sampling day. Each cotton rope was removed from the pen and placed inside a plastic bag and manually squeezed by hand to extract the oral fluids, which were then centrifuged at 100g for 5 minutes and stored at -20°C until qPCR screening for *L intracellularis* DNA.

Fecal samples were prepared by diluting 2 g of feces in 10 mL of phosphate-buffered saline. Then, suspensions were homogenized by vigorous vortexing and later decanted. Nucleic acids were extracted directly from FS and OF supernatants using a nucleic acids purification kit (MagMAX Pathogen RNA/DNA Kit, Thermo-Fisher) on an automated King-Fisher Flex Purification System (Thermo-Fisher) according to the manufacturer's instructions and eluted with 90 µL of nuclease-free water.

Samples were examined for 4 important bacteria known to cause diarrhea in fattening pigs using in-house Biovet finisher pig diarrhea multiplex qPCR (Biovet). The 4 bacteria were *Brachyspira hyodysenteriae*, *Brachyspira hamptonii*, *L intracellularis*, and *Salmonella*. Testing was performed according to the manufacturer's instructions.

A short questionnaire (see Supplementary Materials) was created to obtain site demographics including province, the detection and diagnosis of *L intracellularis* in the past 2 years, the use of *L intracellularis* vaccines before 2021, the strategy of ongoing *L intracellularis* vaccination, the presence of clinical signs of enteric disease, or common endemic diseases (eg, PRRSV, porcine circovirus type 2 [PCV2], and *Mycoplasma hyopneumoniae*). The questionnaire was distributed to herd veterinarians of enrolled sites at the beginning of the study via Microsoft Teams (Microsoft Corporation), completed by the veterinarian at the time of sampling, and returned to investigators over the course of the study. Questions with a response rate < 80% (ie, > 20% missing responses) were excluded from the data analysis.

Statistical analysis

Statistical analyses were performed using R (version 4.2.2).²² Given sample size was based on logistical and budget-related aspects, *post hoc* chi-squared power analysis was conducted based on the number of collected samples (sample size) and the effect size calculated from the probability of detection using G*Power (version 3.1.9.7).²³ The probability of committing a type I error (α) was set at .05. For the power estimation on detecting the effect of sample type (sample-level analysis), FS were used as the proportions of *L intracellularis* DNA positive and negative under the null hypothesis ($p(H_0)$ in G*power) while OF samples were used under the alternative hypothesis ($p(H_1)$ in G*power). Likewise, for the detection of *L intracellularis* vaccination effects, the detection of *L intracellularis* in unvaccinated and vaccinated sites (site-level analysis) was used for determining the $p(H_0)$ and $p(H_1)$, respectively.

Of the total 480 projected samples, 440 (85.7%) were used in the final analysis. Regarding the omitted samples, 29 OF samples collected from the mid-finisher phase did not meet the minimum sample quality (ie, contaminated by feces, insufficient amount of obtained fluids, or

failed internal control after retesting) for *L intracellularis* qPCR (vaccinated sites = 24; unvaccinated sites = 5). In addition, 5 FS samples (vaccinated sites = 2; unvaccinated sites = 3), and 6 OF samples (vaccinated sites = 1; unvaccinated sites = 5) from the late-finisher phase had inconclusive qPCR results and were omitted from the analysis. Statistical power was estimated to be 99.96% at the sample level (total number of samples, $n = 440$) using the contingency table (Table 1) formed by the detection of *L intracellularis* and the specimen type (OF/FS). Similarly, the site-level power (total number of samples, $n = 40$) was estimated to be 93.13% using the contingency table (Table 1) consisting of the detection of *L intracellularis* and the *L intracellularis* vaccination status among sites.

Descriptive statistics are reported as the number of qPCR *L intracellularis*-positive and -negative sites, and proportions of positive and negative samples by sample type and vaccination status including vaccine dosage used and production phase. The *L intracellularis* positivity measured by qPCR was compared between vaccinated and unvaccinated herds by specimen type and sampling phases using Fisher exact test.

Association between specimen type and *L intracellularis* detection

The effect of specimen type (OF and FS) on the detection of *L intracellularis* was investigated by building a multivariable logistic mixed regression model at the sample level using the binomial distribution. The model (Figure 1) consisted of the binary *L intracellularis* detection of each sample as the outcome variable (positive/negative), specimen type (OF/FS) as the fixed effect of interest, and potential confounders (eg, production phase (mid-/late-finisher), *L intracellularis* vaccination status (yes/no), detection of other endemic diseases (yes/no for each disease), detection/clinical observation of *L intracellularis* in the past two years (yes/no), use of *L intracellularis* vaccine prior to 2021 (yes/no), number of pigs in the sample barn (continuous).

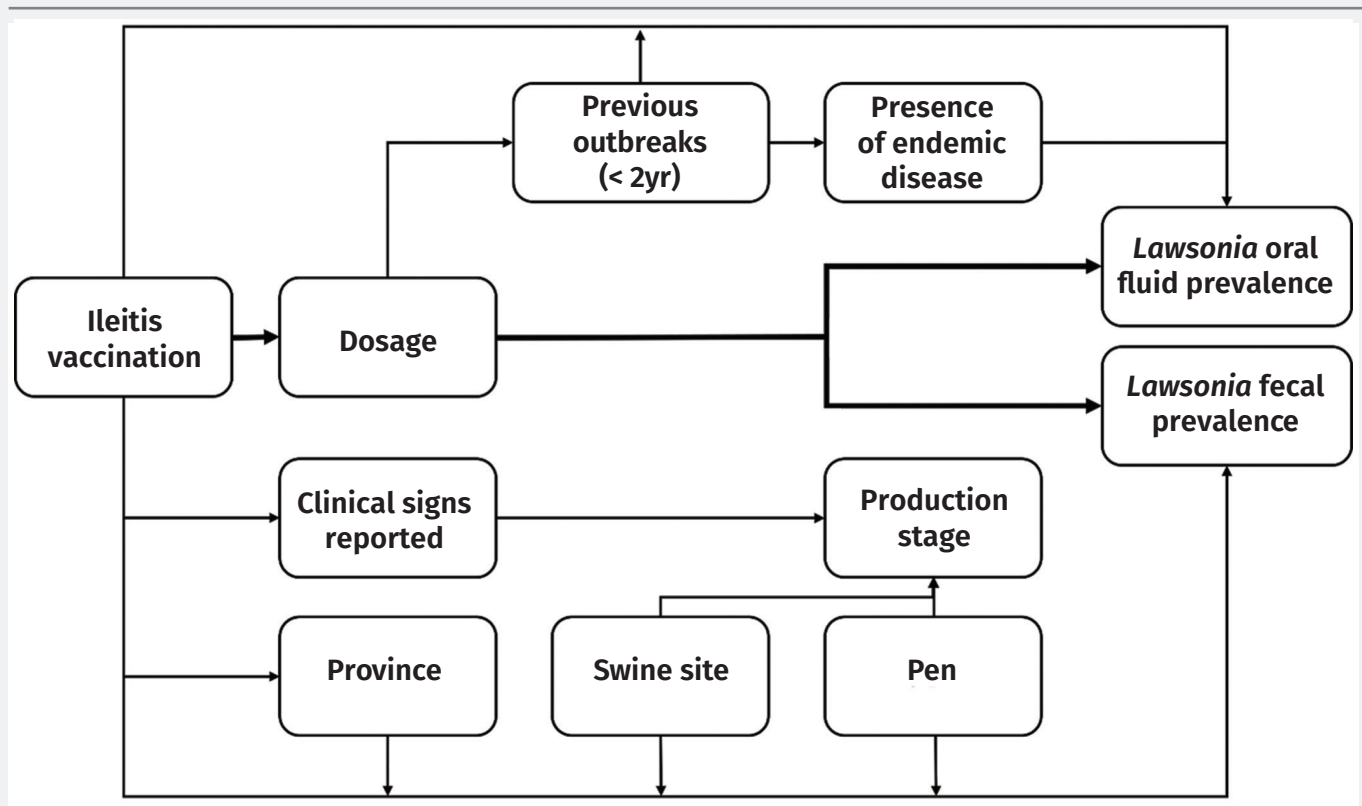
Prior to statistical modeling, potential confounding variables were screened based on pairwise correlation and unconditioned effects on the outcome, ie, the detection of *L intracellularis* DNA, by reporting Cramér's V and constructing univariable models, respectively.

Table 1: Contingency tables for the estimation of statistical power for detecting the effect of sample type (sample-level analysis) and site vaccination status (site-level analysis) on *Lawsonia intracellularis* positivity

Level of effects		<i>L intracellularis</i> DNA detection status		
		Positive, No. (%)	Negative, No. (%)	Statistical power (1-β)*
Sample type	Oral fluids (n = 205)	47 (22.9)	158 (77.1)	99.96%
	Fecal (n = 235)	34 (14.5)	201 (85.5)	
Site <i>L intracellularis</i> vaccination status	Vaccinated (n = 235)	13 (65.0)	7 (35.0)	93.13%
	Unvaccinated (n = 20)	7 (35.0)	13 (65.0)	

* Statistical power estimation was performed using G*Power (version 3.1.9.7).²³

Figure 1: Causal diagram displaying the investigation of relationships between ileitis vaccination protocols, selected random and fixed effects, and oral fluid and fecal sampling prevalence outcomes for *Lawsonia intracellularis* in vaccinated and unvaccinated swine sites. Thicker arrows represent the strongest expected relationship.



Variables with a P value $\geq .2$ in the univariable models were excluded from the multivariable analysis. In addition, for each pair of strongly correlated variables (Cramér's $V > .25$) with significant univariable effects, the one(s) with highest P value in the univariable models were excluded from the multivariable models.

To account for multi-level clustering effects within the dataset, the sampled pen identification, site, and province were included in the model as a nested random effect (pen \subset site \subset province).

Biologically relevant interactions between variables retained in the models were considered. Significance was declared at $P < .05$ and a trend at $.05 \leq P < .10$. The effect of each variable was reported as an odds ratio (OR) with a profile likelihood 95% CI, indicating the fold change of the odds of samples being *L intracellularis* positive. In addition, the intraclass correlation coefficient (ICC) was reported to show the proportion of data variation explained by the random effect term.

Risk factor analysis for *L intracellularis* detection

Potential risk factors associated with the detection of *L intracellularis* in vaccinated and unvaccinated sites were separately assessed at the site level using two Poisson logistic regression models. In particular, the detection risk of *L intracellularis* was estimated as the proportion of positive samples, ie, positive rate, of each site regardless of the specimen type, and was included in both models by assigning the count of positive samples as the outcome and the

total number of collected samples as the offset. The overall detection risk based on all samples was used to increase the sample size at the farm level. Thus, the models were constructed to estimate the proportion of positive OF or FS samples from a farm. Risk factors listed in Figure 1 were screened using the same procedure as described in the previous section. For vaccinated sites, dosage (full, half, and quarter doses) and the use of a booster (yes/no) were included to investigate the effect of vaccination strategy (Table 2). Likewise, the effect of each variable was reported as an incidence rate ratio (IRR) with a profile likelihood of 95% CI, indicating the fold change of sites' *L intracellularis*-positive rates and significant effects were declared as previously described.

Results

For sites actively vaccinating against *L intracellularis*, we found a range of different self-imposed vaccination strategies from the questionnaire, ie, quarter dose with a booster (0.5 mL + 0.5 mL), half dose with no booster (1.0 mL), half

dose with a booster (1.0 mL + 1.0 mL), full dose with no booster (2.0 mL), and full dose with a booster (2.0 mL + 2.0 mL). Additional descriptives of vaccinated sites are found in Table 2.

Overall, 20 of 40 sites (50%) tested positive for *L intracellularis*, of which 65.0% were vaccinated (13 of 20) and 35.0% were unvaccinated (7 of 20; Table 3). Regardless of sampling method, 81 of 440 samples were considered positive (OF: 47 of 205 [22.9%]; FS: 34 of 235 [14.5%]), yielding a mean *L intracellularis* detection risk of 18.7% (Table 3). For mid-finisher pigs, a higher proportion of positive FS was detected in unvaccinated (9 of 57 [15.8%]) compared to vaccinated herds (6 of 59 [10.2%]), whereas a higher proportion of positive OF samples were detected in vaccinated (13 of 38 [34.2%]) compared to unvaccinated herds (10 of 52 [19.2%]; Table 3). For late-finisher pigs, a larger number of positive samples were found for both specimen types in vaccinated (FS: 12 of 60 [20.0%]; OF: 16 of 58 [27.6%]) compared to unvaccinated sites (FS: 8 of 57 [14.0%]; OF: 7 of 59 [11.9%]; Table 3). In addition, only 22.5% (9 of 40)

and 52.5% (21 of 40) of producers responded to the questions regarding use of water (Question 25; Supplementary Materials) and feed medication (Question 27; Supplementary Materials) and were therefore excluded from the data analysis.

For the sample-level model investigating the effect of specimen type on *L intracellularis* DNA detection, the production phase, *L intracellularis* vaccination status, and number of pigs in the sampled barn were screened and accounted for in the Poisson logistic regression model as confounders. Overall, the use of OF sampling yielded a two-fold increase in the odds of detecting *L intracellularis* DNA when compared to FS (OR = 2.36; 95% CI, 1.24-4.49; $P < .01$). In contrast, no significant effects of animal production phase, site vaccination status, and the number of pigs in the sampled pens were found. According to the ICC analysis on the nested random effect, site identification nested within province explained 59% of the data variation whereas the pen identification (nested within site identification and province) and province explained less than 0.01%. Among unvaccinated sites,

Table 2: Demographics and vaccination protocols of *Lawsonia intracellularis* vaccinated swine sites in Canada

Vaccination protocol (dose + booster)	No. of sites	Mean herd size (SD)	Dose structure, mL	Total dosage, mL	Age at administration, d	Age at booster, d	Gastrointestinal signs*	No. sites with ileitis < 2 yr [†]
All vaccinated sites (n = 20)								
1/1 + 1/1	3	3212 (2435)	2.0 + 2.0	4	3	21-42	No	2
1/1	1	102	2.0	2	28	n/a	No	1
1/2 + 1/2	8	2470 (1099)	1.0 + 1.0	2	21-35	42-50	No	1
1/2	3	2520 (3217)	1.0	1	21-35	56	No	1
1/4 + 1/4	5	3162 (1712)	0.5 + 0.5	1	21	42	Yes (1 site)	1
Vaccinated sites positive for <i>L intracellularis</i> (n = 13)								
1/1 + 1/1	2	163 (138)	2.0 + 2.0	4	21	28-42	No	2
1/1	1	34	2.0	2	28	n/a	No	1
1/2 + 1/2	5	323 (241)	1.0 + 1.0	2	21-35	42-50	No	1
1/2	2	334 (250)	1.0	1	24-35	56	No	1
1/4 + 1/4	3	49 (18)	0.5 + 0.5	1	21	21-42	Yes (1 site)	1

* Visible gastrointestinal signs at the time of vaccination.

† Sites with previous outbreaks of ileitis within the last two years.

Table 3: Number of positive and negative *Lawsonia intracellularis* sites and samples per Canadian province, sampling type, vaccination status, and production phase across 20 swine sites and 440 collected samples

<i>L intracellularis</i> positive sites per province					
Province	n	Vaccinated, No. (%)		Unvaccinated, No. (%)	
MB	11	8 (72.7)		3 (27.3)	
ON	8	5 (62.5)		3 (37.5)	
QC	1	0 (0.0)		1 (100.0)	
Total		13 (65.0)		7 (35.0)	

Sample distribution per province					
Province	n	Positive, No. (%)		Negative, No. (%)	
MB	237	45 (19.0)		192 (81.0)	
ON	180	33 (18.3)		147 (81.7)	
QC	23	3 (13.0)		20 (87.0)	
Total	440	81 (18.4)		359 (81.6)	

Distribution per sampling type					
Sample type	Vaccinated, No. (%)		Unvaccinated, No. (%)		P value*
	Positive	Negative	Positive	Negative	
Mid-finisher phase					
OF	13 (34.2) ^a	25 (65.8)	10 (19.2) ^a	42 (80.8)	.14 ^a
FS	6 (10.2) ^b	53 (89.8)	9 (15.8) ^b	48 (84.2)	.42 ^b
Total	19 (19.6)	78 (80.4)	19 (17.4)	90 (82.6)	
Late-finisher phase					
OF	16 (27.6) ^c	42 (72.4)	8 (14.0) ^c	49 (86.0)	.11 ^c
FS	12 (20.0) ^d	48 (80.0)	7 (11.9) ^d	52 (88.1)	.32 ^d
Total	28 (23.7)	90 (76.3)	15 (12.9)	101 (87.1)	

* A Fisher's Exact test was used to determine statistical differences between positive samples in vaccinated and unvaccinated herds by sampling type and production phases. Statistical difference was determined at $P < .05$.

^{a-d} Superscripts specify Fisher's Exact comparison between the number of positive samples from vaccinated or unvaccinated sites. OF = oral fluids; FS = fecal sample.

the detection of PCV2 and the presence of gastrointestinal (GI) signs at the time of sampling showed significant effects on the *L intracellularis*-positive rate, regardless of sample types. Sites positive for PCV2 were estimated to have 4.99 times higher odds of also being positive for *L intracellularis* than PCV2-negative sites (IRR = 4.99; 95% CI, 1.29-20.23; $P = .02$). No effect of PRRSV was observed. Additionally, herds without GI signs at the time of vaccination had 9 times lower odds of also being positive for *L intracellularis* as compared to those herds showing GI signs at the time of sampling (IRR = 0.1; 95% CI, 0.02-0.42; $P < .01$).

A significantly lower positive rate was found in those vaccinating using a full dose with a booster compared to those using a half dose with no booster (IRR = 0.22; 95% CI, 0.06-0.83; $P = .03$). Furthermore, a higher positive rate was estimated for sites that had *L intracellularis* cases diagnosed in the past 2 years (IRR = 3.08; 95% CI, 1.51-6.37; $P < .01$), administering *L intracellularis* vaccines before 2021 (IRR = 9.85; 95% CI, 1.95-179.55; $P = .03$), or for herds positive for *M hyopneumoniae* during the study period (IRR = 4.41; 95% CI, 2.00-10.75; $P < .001$). Province was not included in both models as a random effect due to the overfitting issue. Interaction terms were not included in both models due to the singular fit issue.

Discussion

This study found *L intracellularis* present in 50% of the investigated wean-to-finish swine sites during the mid- to late-finisher phases of which 65.0% of the sites were vaccinated with an inactivated intramuscular vaccine while 35.0% were not. Previously reported prevalence and seroprevalence of *L intracellularis* have varied greatly in herds in both Europe and North America.^{9,12,24,25} However, and in contrast to our study, none of the sites enrolled in those studies were actively administering an *L intracellularis* vaccine. As our study was designed to incorporate vaccinated and unvaccinated swine sites, it is likely that the observed

detection risk will differ from the general swine population. Furthermore, in regard to sample positivity based on sampling methodology, OF yielded a higher risk of *L intracellularis* infection detection compared to FS in vaccinated sites while the opposite was observed in unvaccinated sites. However, a higher proportion of positive samples occurred in vaccinated sites compared to unvaccinated sites during the late-finisher phase regardless of sampling type. It is possible that swine in the late-finisher phase had more opportunities to come in contact with the pathogen, thus enabling a more advanced disease progression to occur compared to younger swine. In addition, circumstantial factors that may have impacted the accuracy of the sampling techniques should not be underestimated or disregarded,¹³ although sampling accuracy was not controlled for in our study. Given the investigative nature and behavior of swine, they could have had the opportunity to interact with both fecal matter present in the pen as well as ropes used for OF sampling, which could act as a vector between the 2 sampling sources as *L intracellularis* naturally transmits between pigs via the oral-fecal route.²⁶

Our models showed that OF sampling had approximately twice the chance of detecting *L intracellularis* compared to FS after accounting for vaccination status and the number of pigs in the sampled herd. These results are in line with previous studies comparing the sensitivity for OF and FS in other infections such as PCV2, PRRSV, porcine parvovirus 3, 5, and 6, and porcine deltacoronavirus.²⁷⁻²⁹ There are also indications for higher sensitivity of OF compared to FS over time for detecting porcine epidemic diarrhea virus.¹⁶ Although the generalized assumption that OF has a higher sensitivity compared to FS can be made from these examples and the results of our study, this may in fact be attributed to specific disease pathogenesis affecting the level of virus shedding. In turn, this may influence detection risks for different sampling techniques and time of sampling and therefore, our results should be interpreted cautiously and on a case-by-case basis. The current study was not designed to make specific inferences about sensitivity and specificity, as we lacked the presence of a validated robust gold standard applied to individual subjects.

Our study found that swine sites without GI signs at the time of sample collection had a 90% decrease in the odds of being

L intracellularis positive. Being an agent of porcine proliferative enteropathy and one of most common causes of diarrhea in swine,³⁰ it is not surprising that sites lacking clinical GI signs in their pigs showed lower odds of harboring swine infected with *L intracellularis*. It has previously been reported that natural gut microbiota changes during weaning may cause younger pigs to be increasingly susceptible to enteric infections and for weaned pigs to be more commonly infected with *L intracellularis* compared to older pigs.³¹ In our study, we found that unvaccinated herds with the presence of a clinical PCV2 diagnosis at the time of sampling increased the odds of detecting *L intracellularis* five-fold compared to herds absent of PCV2. Similarly, herds with a clinical diagnosis of *M hyopneumoniae* increased the odds of being *L intracellularis* positive by more than four-fold.

This study also had important limitations. The sample size may have impacted the study representation and power detecting effects of associated risk factors, especially on a site-level analysis. Additionally, the number of animals that contributed to pooled OF and FS samples was unknown and likely varied, and this could impact the probability of *L intracellularis* detection. Furthermore, missing *L intracellularis* testing results and questionnaire responses may have biased the model estimates and affected study findings, as all clinical diagnoses were self-reported by the herd veterinarians and not independently verified by the research group. Finally, to increase understanding between the associated risk factors of *L intracellularis* detection, a more comprehensive model including movements of pigs, staff, and feed, production data, and long-term health data, and density of commercial swine farms in the region should be implemented in future studies.

The results of this study indicate that the use of OF may have a better *L intracellularis* detection rate when compared to FS. Based on the results of this study, the observed site positivity for *L intracellularis* may be linked to the use of lower amounts than the recommended vaccine dose and pre-existing GI pathogens on site, such as porcine circovirus and mycoplasma pneumoniae. Additional research is recommended to determine sample methodology efficacies and risk factors associated with positive detection of *L intracellularis*.

Implications

Under the conditions of this study:

- Oral fluids may be a useful method for detecting *L intracellularis* in swine.
- Previous health status may impact risks of *L intracellularis* infection.
- Time of sampling may affect OF and FS detection rates.

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Conflict of interest

Drs Van De Weyer and Angulo are currently employed by Zoetis. This project was funded by Zoetis. Zoetis is a global animal health company with a portfolio that includes vaccine development and manufacturing for pets and livestock, including swine.

Disclaimer

Dr Arruda, a member of this journal's editorial board, was not involved in the editorial review or decision to publish this article.

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