An observational study on the prevalence and impact of *Isospora suis* in suckling piglets in southwestern Ontario, and risk factors for shedding oocysts

Andrea Aliaga-Leyton, Emma Webster, Robert Friendship, Cate Dewey, Kevin Vilaça, Andrew S. Peregrine

Abstract — An observational study was conducted to determine the prevalence of *Isospora suis* oocysts in fecal samples from suckling piglets in Ontario, and to evaluate the relationship between the presence of *I. suis* oocysts and diarrhea. Fifty farms and 709 litters of piglets were included in the study. Oocysts were detected on 70% of farms, with 187 litters infected. A litter of pigs that was positive for oocysts was significantly more likely to exhibit diarrhea than a litter that was negative [odds ratio (OR) = 4.0; 95% confidence interval (CI) = 2.8 to 5.8; \( P < 0.001 \)]. Management and housing factors were examined with respect to risk factors for the presence of *I. suis*. Farms that did not use a detergent when cleaning farrowing crates were 10-times more likely to be positive for *I. suis* than those that used a detergent (\( P = 0.007 \)). It was concluded that coccidiosis is a common problem on Ontario swine farms.

Résumé — Une étude observationnelle sur la prévalence et l’impact d’*Isospora suis* chez des porcelets de lait du Sud-Ouest de l’Ontario et les facteurs de risque pour l’excérétion d’ookystes. Une étude observationnelle a été réalisée pour déterminer la prévalence d’ookystes d’*Isospora suis* dans des échantillons de matières fécales provenant de porcelets de lait de l’Ontario et évaluer le lien entre la présence d’ookystes d’*I. suis* et de la diarrhée. Cinquante fermes et 709 portées de porcelets ont été incluses dans l’étude. Des ookystes ont été détectés dans 70 % des fermes et 187 portées étaient infectées. Une portée de porcelets qui avaient des résultats positifs pour les ookystes présentait une probabilité beaucoup plus grande de manifester de la diarrhée qu’une portée qui avait des résultats négatifs [ratio d’incidence approché (RIA) = 4,0; intervalle de confiance (IC) de 95 % = 2,8 à 5,8; \( P < 0,001 \)]. Les facteurs de logement et de gestion ont été examinés en rapport avec les facteurs de risque pour la présence d’*I. suis*. Les fermes qui n’utilisaient pas un détergent lors du nettoyage des cases de parturition avaient une probabilité de 10 fois supérieure d’obtenir des résultats positifs pour *I. suis* que celles qui utilisaient un détergent (\( P = 0,007 \)). On a conclu que la coccidiose est un problème courant dans les fermes porcines de l’Ontario.

(Traduit par Isabelle Vallières)
Introduction

I. suis is a protozoan parasite that reproduces in enterocytes of the small intestine of pigs. Although the parasite was identified for the first time in pigs in 1934 (1), it was not until the mid-1970s that coccidiosis was recognized as a clinical disease problem in pigs (2,3). Sanford and Josephson (4) demonstrated that I. suis could be commonly identified in the gut tissue of piglets with enteric disease submitted to a veterinary diagnostic laboratory in Ontario (4). However, no further studies regarding piglet coccidiosis in Ontario have been published since that time. The many changes in housing and husbandry that have taken place in the past 30 y may have led to a reduction in the prevalence and impact of I. suis. In Europe and elsewhere, however, this parasite is today recognized as a frequent and important cause of diarrhea and uneven weight gains in suckling piglets (5,6).

In most countries, the only drug that has been licensed for prevention of coccidiosis in pigs is toltrazuril (Baycox; Bayer AG, Kiel, Germany). The administration of 20 mg toltrazuril per kg bodyweight (BW) as a single oral dose in the first week of life provides a significant economic benefit, even when coccidial infections are subclinical (7). Toltrazuril has never been licensed for use in Canada, but in problem herds the drug was used under veterinary supervision with an investigational new drug submission until 2005. In June 2005, the investigational new drug submission was rescinded, which stopped the sale of Baycox. As a result, the Canadian swine industry was without an effective therapeutic approach for cases of piglet coccidiosis.

The objectives of this study were to determine the prevalence of I. suis oocysts in fecal samples from suckling piglets in Ontario, to determine if the presence of oocysts is associated with the occurrence of diarrhea and/or reduced growth rates in suckling piglets, and to identify housing and management risk factors associated with I. suis infections.

Materials and methods

A cross-sectional study was conducted between May and August 2006 on a convenience sample of 50 farms located in southwestern Ontario. Practicing veterinarians in Ontario provided the names of swine producers who were willing to participate in the study. All farms were visited on 1 occasion (n = 2 researchers). On each farm, up to 10 litters were chosen in each of 2 age groups: 7 to 15 d of age and 16 to 21 d of age. On small farms, when there were 10 or fewer litters in each age category, all available litters were sampled. On larger farms, with more than 10 litters from which to select, litters were purposely chosen in order to achieve an even distribution throughout the farrowing room(s). Stratified random sampling was used to select farrowing crates within each age group (8). Pooled fecal samples were collected from each litter that was chosen for the study. This was done by picking up individual piglets and using anal stimulation to cause defecation. In addition, if littermates were observed defecating during this collection period, the freshly voided feces were also collected. Three to 5 samples from each litter, approximately equal in size, were pooled and mixed. Piglets within a litter were conveniently, rather than randomly, chosen for fecal sampling. The pooled samples were placed in a container that was identified by litter and farm, and placed in a cooler for transport to the laboratory.

A modified Cornell-Wisconsin centrifugal floatation method was used to examine fecal samples for coccidia oocysts (9). In brief, 2.0 g of feces were mixed with 15 mL of water and passed through a 1-mm sieve. The filtrate was spun in a 15 mL centrifuge tube at 396 × g for 5 min. After removal of the supernatant the tube was half filled with sucrose solution (specific gravity = 1.27 to 1.33) and the contents mixed well. Sucrose solution was then added until the tube was 3/4 full, mixed well, and additional sucrose solution added until a meniscus was created onto which a 22 × 22-mm coverslip could be applied. The tube was then centrifuged as before, using freely rotating tube holders, after which the coverslip was carefully removed and placed on a microscope slide for examination at 400×. Samples were defined as infected by the observation of 1 or more I. suis oocysts. Oocyst determination was also carried out in a semi-quantitative manner by observing the number of oocysts under the coverslip; the minimum level of detection of this method is 0.8 oocysts per g of feces.

In all litters aged 16 to 21 d, the ages of the piglets were recorded; individual weights were obtained to 0.1 kg increments using a spring scale (Pelouze; model 4010; Sunbeam Products, Aurora, Illinois, USA). The consistency of feces at the litter level was recorded using freshly voided samples from 3 to 5 piglets and the following scoring system: 0 for normal, 1 for loose pasty, and 2 for watery feces.

A 2-page survey was completed at the time of the farm visit. The researchers recorded observations regarding the housing and facility design, and the farm manager or owner was asked questions regarding husbandry and management. Questions on the survey primarily dealt with the farrowing rooms and management of the nursing sows and their litters. The overall number of sows, the number of farrowing places, and number of farrowing rooms were recorded, as well as average weaning age. Farrowing crate design, particularly details regarding flooring, were noted. In addition, questions were asked about hygiene, such as cleaning procedures and pig flow. There were also questions regarding sow feed and creep feed and if medication was used. In particular, farmers were asked if they used coccidiostats and if diarrhea was a problem in suckling pigs. A copy of the survey can be obtained from the authors.

This study had the approval of the University of Guelph Animal Care Committee and was conducted following the guidelines of the Canadian Council on Animal Care.

Statistical analysis

Data were recorded on each visit using a standardized form, then transferred to an electronic spreadsheet program for editing and manipulation (Microsoft Excel 2003; Microsoft corporation, Redmond, Washington, USA). All analyses were conducted using STATA intercooled version 9.2 (Stata Corporation, College Station, Texas, USA). The infection status was defined at the litter level as a dichotomous variable, and also as a categorical variable based on the quantitative oocyst count. Initially, oocysts per slide were counted. This value was then transformed...
into oocysts per g (OPG), using the following mathematical equation:

$$\text{OPG} = \left( \frac{a}{b} \right) \times 1.6$$

where: $a =$ total number of oocysts observed per slide and $b =$ g of feces used for the technique (9). A scale was created from 0 to 4 (category 0: no oocysts observed, category 1: 0.8 to 80 OPG, category 2: 80.8 to 160 OPG, category 3: 160.8 to 800 OPG, and category 4: > 800 OPG). These were arbitrary thresholds used for this study. A farm was considered positive for *I. suis* if 1 oocyst was identified in any of the pooled samples examined.

Since each farm was visited only once during the study period, all weaning weights were standardized to 21 d of age. Within each farm, weight was regressed on age using linear regression. The within-farm slope, as measured by the regression coefficient, was used to adjust the weight to 21 d of age for each pig. The estimated differences between average-adjusted 21-day weights by herd *I. suis* status were compared using a 2-tailed *t*-test.

In evaluating risk factors, the farm infection status was treated as a dichotomous variable. A simple logistic regression model was used to evaluate the association between farm infection status and each putative causative variable. Herd size was categorized by units of 20 farrowing crates. Risk factors with multiple categories, such as farrowing crate floor type, were reanalyzed using logical groupings. For example, in the case of farrowing crate flooring, the different types were examined individually and finally grouped into either all slatted or crates of farrowing crate flooring, the different types were examined.

**Results**

Farm size varied from 30 to 1700 sows [mean = 411, standard deviation (s) = 384]. The mean number of farrowing rooms was 5 per farm, with a minimum of 1 and a maximum of 29. Crates per room varied from 5 to 60 with a mean of 19. The total number of crates per farm was 81, $s =$ 68 (min = 16, max = 290).

A total of 709 litters and 3372 piglets on 50 farms were included in the study. On average, 14 litters were sampled per farm (min = 2, max = 20). *Isospora suis* oocysts were detected on 70% (35/50) of the farms, with 26.4% (187/709) of litters infected. With respect to the level of shedding of oocysts, 90 litters were classified as category 1 (0.8 to 80 OPG), 24 litters were in category 2 (80.8 to 160 OPG), 34 litters were in category 3 (160.8 to 800 OPG) and 39 litters were in category 4 (> 800 OPG).

Of the 2857 piglets aged 16 to 21 d that were weighed, the mean and standard deviation of weights on the day of sampling were 5.9 kg and $s =$ 1.6 kg (min = 1.2 kg, max = 12.1 kg). The mean standardized 21-d weight for the piglets on farms where *I. suis* oocysts were detected was 6.3 kg, $s =$ 1.0 kg, while it was 6.7 kg, $s =$ 1.2 kg for piglets on *I. suis*-negative farms. The difference of 0.4 kg in the mean 21-d weights was not significant ($P = 0.19$). None of the farms weaned their piglets at less than 15 d of age; 8% (4/50) of the farms weaned their piglets at 15 to 17 d of age; 44% (22/50) weaned at 18 to 21 d of age; 14% (7/50) weaned at 22 to 25 d of age; 24% (12/50) weaned at 26 to 29 d of age; and 10% (5/50) of the farms weaned their piglets at older than 29 d of age.

Flooring type was categorized as completely solid, partially solid, perforated floor except creep area, and completely perforated. The proportion of farms with each type of floor is presented in Table 1. Of the 35 *I. suis*-positive farms, 8 utilized completely slatted flooring in the farrowing crate while 27 farms had at least some flooring that was solid. Among the 15 non-infected herds, 6 used completely slatted flooring and 9 used some solid flooring. There was no significant difference between completely slatted flooring and all other types of flooring with respect to the *I. suis* status of farms ($P = 0.3$).

Flooring material varied from farm to farm, and often combinations of materials were used in the same crate. Plastic-coated metal and moulded plastic were the most common types of material for perforated flooring (36 farms). Stainless steel slats were used on 12 farms, and cast iron was used on 29 farms. Concrete was present as flooring material in the farrowing crate on 16 farms, and 13 of these were *I. suis*-positive farms. However, concrete flooring was not associated with *I. suis* infection ($P = 0.2$).

Only 52% (26/50) of the farms used a strict all-in/all-out system for operating the farrowing rooms, but 22 farms that did not use an all-in/all-out flow did wash the crates between farrowings. Two farms (4%) did not routinely clean farrowing crates between farrowings. Crates were left empty for: < 12 h, 12 to 24 h, 25 to 48 h, and > 48 h on 38%, 24%, 36%, and 2% of the farms, respectively.

High-pressure washing of the farrowing crate environment was used on 66% (33/50) of the farms with 22 of these farms using hot water or steam. Detergent was routinely used as part of the cleaning procedure on 34% (17/50) of the farms, and

<table>
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</tr>
</thead>
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</tr>
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<td>30 (60)</td>
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<td>Completely perforated</td>
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### Table 1. Type of floor of the farrowing crates on 50 Ontario farms

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### Table 2. Factors associated with positive *Isospora suis* farm status for 50 Ontario farms in final multivariable model

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds ratio</th>
<th>Standard error</th>
<th>P-value</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergent use</td>
<td>0.100</td>
<td>0.086</td>
<td>0.007</td>
<td>0.018 to 0.538</td>
</tr>
<tr>
<td>Farmer-reported diarrhea</td>
<td>0.128</td>
<td>0.109</td>
<td>0.016</td>
<td>0.024 to 0.683</td>
</tr>
</tbody>
</table>

*In pigs up to 21 d old.*
disinfectant was used on 78% (39/50) of the farms. Farms that reported the use of detergent were only 1/10th as likely to be *I. suis* positive as farms that did not report the use of detergent (OR = 0.100; 95% CI = 0.018 to 0.538; *P* = 0.007) (Table 2). Other hygiene-related factors such as the use of disinfectant and all-in/all-out flow were not associated with infection status.

There was an association between litters being positive for *I. suis* oocysts and litters having diarrhea (OR = 4.0; 95% CI = 2.8 to 5.8; *P* < 0.001). However, the reverse relationship was found with regard to the survey question concerning the presence of diarrhea on farms in suckling piglets; farms where the interviewee answered that diarrhea was a serious problem were less likely to be positive for *I. suis* (OR = 0.128; 95% CI = 0.024 to 0.683; *P* = 0.016) (Table 2). Diarrhea was reported as a common problem in suckling piglets on 19 farms (38%), although only 1 farm used a coccidiostat (diclazuril, Clinafox; Intervet Schering-Plough) in an extra-label manner. Among the 50 producers, 10% reported that diarrhea usually started at 24 h of age, 34% said that diarrhea started at 2 to 4 d of age, 16% said that diarrhea started at 5 to 7 d of age, 42% said that diarrhea started in the second week of life, and 8% said that diarrhea started at 3 wk of age. Seven respondents claimed that diarrhea occurred at 2 different ages.

Most producers (78%) treated diarrhea with antibiotics and generally believed their treatment was effective. Only 1 interviewee reported that the diarrhea on their farm was non-responsive to treatment. Producers were also asked if sows were medicated or vaccinated prior to farrowing. On 13 farms the gestation sow feed was medicated with an antibiotic. Eighty-two percent (41/50) of the farms vaccinated their sows prior to farrowing; 34 farms used an *E. coli* vaccine, 5 farms used a vaccine for rotavirus, 4 farms used a coronavirus vaccine, and 12 farms used a vaccine for *Clostridium perfringens*.

As mentioned, in the multi-variable analyses, factors associated with a decreased risk of being an *I. suis*-positive farm included using detergent, and the farmer recognizing diarrhea in pigs under 3 wk of age as a problem (Table 2). The diagnostics conducted on the multi-variable model indicated that the data fit the model well.

### Discussion

This cross-sectional study detected *I. suis* on 70% of the swine farms in the study population. The herds were not randomly chosen and therefore the prevalence determined in this study cannot be extrapolated to the general population of swine herds in southwestern Ontario. However, the high prevalence of *I. suis*-positive farms is very similar to the levels reported in a study in Germany, Austria and Switzerland (76% of farms positive) (5), in Poland (66% of farms positive) (10), and in Brazil (82% of farms positive) (11).

The association observed in the present study between *I. suis*-positive litters and diarrhea is consistent with reports from diagnostic laboratories that commonly find *I. suis* present in the intestinal tissues of suckling piglets with enteric disease. In a study by Driesen et al (12), *I. suis* oocysts were present in 53.8% of samples from diarrheic piglets, compared with enterotoxigenic *E. coli* which were present in 18.2% of the samples, and rotavirus which was found in 16.9% of the samples (12).

Our data suggest that *I. suis* does not have an impact on growth up to 21 d of age. As such, the finding is inconsistent with work in Belgium (7). Theoretically, this could be due to the occurrence of *I. suis* at lower levels in Ontario swine herds compared to those in Belgium. However, it should be noted that this study was not designed to make a definitive conclusion about the association of *I. suis* infection with growth up to 21 d of age; pig weights were determined on only 1 occasion. Furthermore, an adjusted 21-day weight was calculated rather than measuring the actual 21-day weight of the pigs. Also, weaning weight differed by farm. In addition, it was not possible to account for confounding factors that might have affected weaning weights on each farm. For example, some farms could have had a heavier mean weight at a given age than others due to differences in management, feed, litter size, or genetics. In order to determine the true impact of *I. suis* infection on growth rate it is necessary to conduct a longitudinal study controlling for these confounding factors.

Results from the survey and diagnostic data indicated that *I. suis* was more likely to be found on farms where diarrhea in suckling pigs was not perceived by producers to be a common problem compared to farms where diarrhea was perceived as a common problem. In contrast, a significant correlation was found between the presence of *I. suis* oocysts and diarrhea within litters. One can speculate that if a producer’s pigs routinely show clinical evidence of diarrhea, she or he may become habituated to the diarrhea and may not perceive this to be a problem.

A common response amongst farmers was that suckling piglet diarrhea on their farm started at 2 to 4 d of age (17/50), which is likely too early to be caused by *I. suis* because the prepatent period is 4 to 7 d (13). It is likely that on most farms a variety of enteric pathogens are present and that infection with 1 agent may contribute to infection and impact of a second pathogen, so that even in herds where *E. coli* or *Clostridium* are present and causing clinical disease, control of coccidiosis may be helpful (14,15).

The use of detergent in the cleaning process reduced the likelihood of a farm being positive for *I. suis*. A similar finding was observed in a study of *Cryptosporidium* prevalence in dairy calves (16). Detergents are extremely important in the cleaning process on pig farms because they help to break down fat and biofilms (17). With regard to coccidiosis prevention, in all likelihood the physical removal of organic matter is more important than the final step of disinfection, as sporulated oocysts of *I. suis* are resistant to most disinfectants (5,18). This is consistent with the lack of association with use of a disinfectant that was observed in this study.

It is generally accepted that concrete flooring tends to be porous and therefore difficult to clean. As a result, concrete flooring has been associated with an increased risk of coccidiosis (19). However, neither the study by Mundt et al (5) nor the work described here revealed an association between the type of flooring in the farrowing crate environment and risk of *I. suis* infection. Possibly a much larger study designed to examine this particular aspect may be necessary before the role of flooring can
be ruled out as an important risk factor for *I. suis* infection. The
difficulty in interpreting the findings of this trial with regard to
flooring type is that there were many different types of floor-
ing designs and materials used on the 50 farms in the study. It
was therefore necessary to group all the solid and partially solid
floor types together in order to compare this floor type with
completely perforated flooring.

Overall, this study demonstrated that *I. suis* oocysts were
present in the feces of suckling piglets on many Ontario farms
and that the presence of oocysts was associated with diarrhea at
the litter level. The work therefore suggests that coccidiosis is
likely a common problem on Ontario pig farms.

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**References**