Isospora suis infection and its association with postweaning performance on three southwestern Ontario swine farms

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Summary

Objective: To determine the association between *Isospora suis* infection and weight gain in pigs up to 8 weeks of age.

Materials and methods: A prospective cohort study was conducted on a convenience sample of three swine farms in Ontario. Fecal samples were collected from randomly selected piglets from each of 72 litters and examined for oocysts using centrifugal floatation. Piglet weight was recorded six times, during the first, second, third, fourth, fifth, and eighth weeks of life. If one or more suckling piglets from a litter were shedding *I suis* oocysts, then the litter was classified as infected. A linear mixed model was used to examine the effect of infection on weight gain.

Results: *Isospora suis* infection during suckling was associated with lower weights of pigs at the end of the nursery stage (average 62 days of age). Pigs from infected litters were 1.4 kg lighter than pigs from non-infected litters (95% CI, 1.1-1.8 kg; $P < .001$).

Implications: Infection with *I suis* during the suckling period can have an impact on postweaning performance. Thus, calculations of the economic impact of coccidiosis need to include the effect of slower growth rates in the first few weeks after weaning, as well as a reduction in suckling pig performance.

Keywords: swine, *Isospora suis*, coccidia, growth rate, nursery

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Resumen - La infección de *Isospora suis* y su relación con el desempeño post destete de cerdos en tres granjas porcinas del suroeste de Ontario

Objetivo: Determinar la relación entre la infección de *Isospora suis* y la ganancia de peso en cerdos hasta la semana 8 de edad.

Materiales y métodos: Se realizó un estudio prospectivo de cohorte en tres granjas porcinas en Ontario. Se colectaron muestras fecales de lechones seleccionados al azar de 72 camadas y se analizaron en busca de oocistos utilizando flotación centrífuga. Se registró el peso de cada lechón seis veces, durante la primera, segunda, tercera, cuarta, quinta, y octava semanas de vida. Si uno ó más lechones lactantes de una camada estaban eliminando oocistos de *I suis*, entonces la camada se clasificó como infectada. Se utilizó un modelo linear mixto para examinar el efecto de la infección sobre la ganancia de peso.

Resultados: La infección de *Isospora suis* durante la lactancia se relacionó con menores pesos en los cerdos al final de la etapa de destete (62 días de edad en promedio). Los cerdos de camadas infectadas fueron 1.4 kg más ligeros que los cerdos de camadas no infectadas (95% CI, 1.1-1.8 kg; $P < .001$).

Implicaciones: La infección de *I suis* durante el periodo de lactancia puede tener un impacto en el desempeño post destete. Por lo tanto, los cálculos del impacto económico de la coccidiosis necesitan incluir la reducción en el índice de crecimiento en las primeras semanas después del destete, así como una reducción en el desempeño de los cerdos lactantes.

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Résumé - Infection par *Isospora suis* et son association avec les performances en période post-sevrage sur trois fermes porcines du sud-ouest de l'Ontario

Objectif: Déterminer l'association entre l'infection par *Isospora suis* et le gain de poids chez les porcs jusqu'à l'âge de 8 semaines.

Matériels et méthodes: Une étude prospective de cohorte a été menée sur un échantillon de convenance sur trois fermes porcines en Ontario. Des échantillons de fèces ont été prélevés de porcs sélectionnés au hasard de chacune de 72 portées et examinés pour la présence d'oocystes par flottaison avec centrifugation. Le poids des porcelets a été enregistré six fois, soit à la première, deuxième, troisième, quatrième, cinquième et huitième semaine de vie. Si un porcelet à la mamelle ou plus d'une portée excrétait des oocystes d'*I suis*, la portée était alors classée comme infectée. Un modèle linéaire mixte a été utilisé pour examiner les effets de l'infection sur le gain de poids.

Résultats: L'infection par *I suis* au cours de la période d'allaitement était associée à des porcs de poids inférieur à la fin de la période en pouponnière (62 jours d'âge en moyenne). Les porcs provenant des portées infectées étaient plus légers de 1.4 kg comparativement aux porcs provenant de portées non-infectées (IC de 95%, 1.1-1.8 kg, $P < 0.001$).

Implications: L'infection par *I suis* durant la période d'allaitement peut avoir un impact sur les performances en période post-sevrage. Ainsi, le calcul de l'impact économique de la coccidiose doit inclure les effets des performances de croissance ralenties durant les premières semaines après le sevrage, aussi bien que la réduction des performances chez les porcelets à la mamelle.

In 2006, a study[1] was carried out on 50 farms in southwestern Ontario to determine the prevalence and impact of *Isospora suis* in piglets 7 to 21 days of age. Oocysts of *I suis* were found on 70% of farms, and litters infected with *I suis* were four times more likely to have diarrhea than non-infected litters. In addition, mean standardized weaning weight in *I suis*-positive farms tended to be lighter than that in non-infected farms.[1] These findings are consistent with results of studies from other countries that report that *I suis* is a common problem in suckling pigs and of economic concern.[2-4] It is possible that *I suis* infection is more important than these studies reveal. Piglets infected with *I suis* develop intestinal damage, including villous atrophy,[3,5-11] and this may occur near the time of weaning. Few studies have examined the effect of *I suis* on weanling pig performance, and yet *I suis* infection could be very important with respect to growth rate and enteric health of the newly weaned pig.

The primary objective of this study was to determine whether piglets derived from litters infected by *I suis* grow more slowly in the postweaning period than piglets from non-infected litters.

Materials and methods

The research protocol was approved by the Animal Care Committee, University of Guelph.

Study One

A pilot study was performed on a farm with a known *I suis* problem[1] to determine a practical and optimal fecal-sampling protocol for Study Two. This farm had participated in a previous study in which fecal samples were collected from suckling piglets and clinical observations regarding diarrhea were recorded. A high prevalence of samples containing oocysts had been noted. For the present study, on a single visit, fecal samples were collected from 188 pigs in 29 litters. All pigs were individually identified. A random-number generator was used to select pigs 5 to 56 days of age for sampling. The selection process was restricted in that at least two pigs were selected for fecal sampling from each litter or pen. All fecal samples were examined individually for *I suis* oocysts using a modified Cornell-Wisconsin technique, a sucrose-centrifugal floatation method.[12]

Data were recorded using a standardized form and then transferred to an electronic spreadsheet program for editing and manipulation (Microsoft Excel 2003; Microsoft Corporation, Redmond, Washington). Data were then transferred to a statistical software program, (Intercooled Stata, version 9.2; Stata Corporation, College Station, Texas), and descriptive statistics were generated.

Study Two

Prospective cohort studies were performed on three swine farms (F2, F3, and F4) selected by convenience and purposive sampling.[13] Selection of farms was based on known *I suis* status and proximity to Guelph. Sample size was calculated to find a difference of 5% in the weight of *I suis*-infected pigs and non-infected pigs at 8 weeks of age. The same variation in weight was assumed for both groups. A standard deviation (SD) of 1.63 kg, and mean weights of 16.23 kg and 15.43 kg, respectively, were used in the estimation of sample size for the unexposed and exposed groups at 8 weeks of age. These numbers were based on results of a previous trial performed in similar Ontario herds.[14] For this calculation, a power of 80% and a significance level of .05 (two-sided) were used, with an intraclass correlation coefficient (ICC) (rho) of 0.25 using three piglets per litter. On the basis of these assumptions, the sample size required to show a significant difference was estimated to be 66 litters. On Farm F2, only 10 litters were available during the study period, so the other two farms provided approximately 30 litters each. The total number of litters was 72 and the number of pigs was 685 for the study.

The study period was May 18 to September 5, 2007. Each farm was visited 1 day per week, and always the same day. No cross-fostering occurred during the time the piglets were on this study. All piglets within the selected litters were identified at approximately 1 week of age using numbered ear tags. Age was recorded from the farm records, and each pig was individually weighed during its first, second, third, fourth, fifth, and eighth weeks of life. On Farms F3 and F4, weights at the first to third weeks were obtained using a postal scale, which measured in 0.1-kg increments (Pelouze, model 4010; Sunbeam products Inc, Aurora, Illinois). Weights at the fourth, fifth, and eighth weeks on these farms were obtained with a mobile scale, which also measured in 0.1-kg increments (Model 75; Reliable Scale Corporation, Calgary, Alberta, Canada). On the third farm, F2, weights at the first to third weeks were obtained using a similar postal scale, but weights at the fourth and fifth weeks were obtained with an electronic scale that measured in 0.01-kg increments (GSE 450; Central Carolina Scale, Sanford, North Carolina), and weights at the eighth week were obtained with a larger scale that measured in 0.1-kg increments (GSE 550; Central Carolina Scale). All weight data were recorded to 0.1 kg accuracy.

Fecal samples were collected from three randomly selected pigs in each litter. A computerized random-number generator was used to select pigs within each litter when they were approximately 2 weeks of age. Sampling was repeated from the same pigs at the third and fifth weeks of life. All samples were analyzed individually using a modified Cornell-Wisconsin centrifugal floatation method that uses 2 g of fresh feces.[12] This procedure has a minimum level of detection of 0.8 oocysts per g of feces. This level of detection is a theoretical limit determined after correcting for the loss of parasite eggs that occurs with this method.[12]

A score was assigned to categorize the consistency of each fecal sample: 0 = normal, 1 = pasty, and 2 = watery feces. Fecal scoring was performed by three people. Additional information, including sow parity, litter size, and birth date were recorded from farm records. In the case of five litters at Farm F3, birth dates were estimated on the basis of the existence of umbilical cords and the size of the piglets, because the farm records were incomplete.

A Woolf test[15] was used to compare the occurrence of watery diarrhea in pigs in *I suis*-infected and non-infected litters.

Data were collected with no management intervention by the researchers, and clusters (farm level, litter level, and pig level) were controlled for in a model in order to predict fixed effects across farms, litters, and pigs.

The individual weights of pigs up to 62 days of age were regressed on putative fixed and random effects using a linear mixed model to determine the association of *I suis* infection at the litter level with body weight.[16,17] Fixed effects considered in the model were farm identification, age of pig, and quadratic for age (at six points of time which coincided with the times when they were weighed), sex of pig, sow parity, number of piglets per litter, and *I suis* infection status at the litter level. Diarrhea was not included in the model, since it was thought that some or all of the effect of *I suis* on low body weight might be mediated through diarrhea. A litter was considered infected if at least one *I suis* oocyst was found in a sample from at least one of the three randomly selected suckling piglets sampled during their second, third, and fourth weeks of life. Positive and negative animals were defined at the litter level (a time-invariant variable); information on *I suis* infection at approximately 5 weeks of age was not used for the analysis performed with the mixed model, since at 5 weeks of age piglets were no longer housed with their littermates. Weight was regressed on age and the quadratic term for age to determine whether the relationship was linear or curvilinear. Also, a Lowess smoother graphic was used to visualize the association between weight and age. Random effects included were litter and pig as a random intercept, and pigs nested within litters within farms.

Outliers and potentially influential points were examined visually by plotting residuals against fitted values at the different levels. Models were refitted after deletion of potential influential observations, and parameters were compared. Normality and homoscedasticity of the residuals at the different levels were tested visually. As a result, the outcome “body weight” was transformed to natural logarithm of weight expressed in kg, in order for the residuals to appear normally distributed and have equal variance distribution; the final model was again tested for normality and homoscedasticity. The choice of the best model was based on the number of observations, covariates significant at $P < .05$ on the basis of the z-test reported for the mixed-model, and on the Akaike Information Criterion (AIC).

Results

Study One

In total, 188 fecal samples were collected (n = 2, 26, 31, 21, 31, 23, 18, and 36 from pigs approximately aged 1, 2, 3, 4, 5, 6, 7, and 8 weeks, respectively). Among the 188 fecal samples, 34 were positive for *I suis* oocysts (Figure 1). In 20 litters of pigs in the farrowing room, all samples were negative; in six litters, all pigs selected tested positive; and in three litters, a combination of positive and negative pigs was found. Sixty-five percent of all positive samples were from piglets aged 15 to 24 days. No piglets were detected infected at < 15 days of age or at > 40 days of age. The mean number of oocysts per g of feces for positive piglets aged 15 to 24 days was 390, median 106.8 (95% CI, 121-658), while the mean for pigs at 25 to 40 days of age was 41, median 26.4 (95% CI, 13-68).

Study Two

The proportions of litters infected with *I suis* were 4 of 10 (40%), 18 of 30 (60%), and 27 of 32 (84.4%) for farms F2, F3, and F4, respectively. The numbers of positive fecal samples per visit per farm are presented in Table 1.

At approximately 3 weeks of age, litters infected with *I suis* were compared to non-infected litters with respect to the occurrence of watery diarrhea in sampled

Figure 1: Study One: Proportion of pigs shedding *Isospora suis* oocysts from 1 to 8 weeks of age on one farm in southern Ontario. During one visit, fecal samples were collected from randomly selected pigs aged 5 to 56 days; a minimum of two pigs were sampled from each litter or pen. Fecal samples were examined individually using a modified Cornell-Wisconsin centrifugal floatation technique. Numbers above bars denote the total number of samples examined. Oocysts were detected in pigs 15 to 40 days of age.

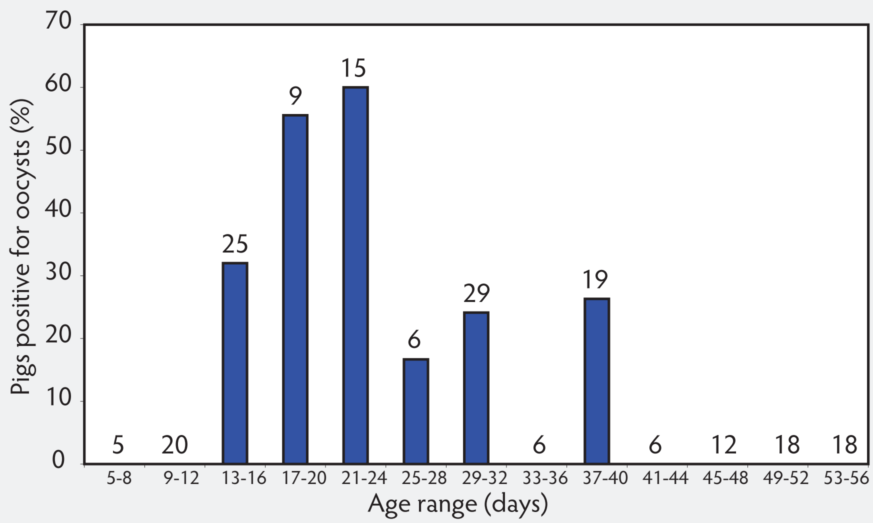


Table 1: *Isospora suis* status of pigs sampled longitudinally at different ages on three farms (F2, F3, and F4) (Study Two)*

Farm	Age of pigs		No. of pigs positive for *I suis* oocysts/no. sampled (%)
	Weeks	Days	
F2	2	12-18	3/30 (10.0)
	3	19-25	7/30 (23.3)
	5	33-39	7/30 (23.3)
F3	2	11-22	42/89 (47.2)
	3	18-29	32/86 (37.2)
	5	32-43	41/85 (48.2)
F4	2	11-14	41/96 (42.7)
	3	18-21	48/93 (51.6)
	5	32-35	29/87 (33.3)

* Fecal samples were collected from three randomly selected pigs per litter at 2 weeks of age; the same pigs were sampled at 3 and 5 weeks of age. All samples were analyzed individually using a modified Cornell-Wisconsin centrifugal floatation method.

individual piglets (fecal score 2 compared to piglets with a fecal score of 0). A total of 21 piglets had watery diarrhea at approximately 3 weeks of age, and 19 of these animals were from *I suis*-positive litters. The odds of piglets having watery diarrhea was 7.34 times higher in infected litters than in non-infected litters (OR = 7.34; 95% CI, 1.63-32.97; Woolf test, $P < .01$). Watery diarrhea was not detected in piglets younger than 3 weeks of age.

Weight measurements were obtained for 685 pigs from 72 litters. The number of piglets per litter varied from four to 13, with a mean of 10, SD = 1.9 piglets per litter. "Sex of the pig" and "number of pigs per litter" were not included in the final model since for these factors, *P* was > .05. A second reason for not including the variable "number of pigs per litter" was because, after comparing the final model (nested) with a model including number of piglets per litter (full), the AIC was smaller for the final model (nested).

The association between pig weight at approximately 8 weeks of age and *I suis* infection at the litter level is presented in Table 2. From the final mixed model, there were 72 litters and 678 piglets nested within the litters at the beginning of the study. For the 678 piglets, there was an average of 5.7 observations per pig with a maximum of six observations (total of 3837 observations), which corresponded to the six occasions when weight information was obtained.

The interaction between age and *I suis* infection implies that the growth rate over time differs by *I suis* status. Although pigs are expected to increase in body weight with age, the rate of increase changes according to whether a pig comes from an *I suis*-positive or *I suis*-negative litter. Pigs from an *I suis*-positive litter gain less weight as they age than pigs from *I suis*-negative litters.

Figure 2 illustrates the predicted weight of pigs over time as determined by the final model. This model predicted that at 8 weeks of age, a pig from an *I suis*-positive litter will weigh 1.4 kg less than a pig from a negative litter (95% CI, 1.1-1.8 kg; $P < .001$). The ICC for weights of the same pig was 0.77, and the ICC for weights of different pigs in the same litter was 0.24.

Discussion

Most studies which have examined the prevalence and impact of *I suis* infection have concentrated on suckling piglets, primarily at 7 to 21 days of age. Age resistance to infection does occur, so that the clinical signs of infection are more severe at 7 to 14 days of life than at 3 or 4 weeks of age (as reviewed by Worliczek et al[18]). As a result, it is possible that, because of the milder nature of disease in the older pig, this age group has generally been ignored. In Study One, *I suis* oocysts were found in the feces of piglets as old as 40 days, with peak shedding occurring at approximately 17 to 20 days. This time of peak shedding was approximately the time of weaning on the study farm, and suggests that, in this herd, the greatest impact of coccidiosis might occur in the early postweaning stage. Damage to the intestinal lining and villous atrophy caused by meronts or gamonts of *I suis* may last for up to 9 days.[19] Unfortunately, weaning is also a time when the gut undergoes dramatic changes because of the sudden loss of passive protection from milk and the change in gut flora as a result of dietary changes. This pilot study therefore helped confirm that a trial to examine the impact of *I suis* on postweaning performance was warranted. The results indicated that examination of fecal samples for *I suis* beyond 5 weeks of age was not necessary, since 65% of all positive samples were detected before 24 days of age, and no positive samples were found beyond 40 days of age. Thus, a protocol of sampling at approximately 2, 3, and 5 weeks of age was a practical regimen for determining the *I suis* infection status of a farm, but only at 2 and 3 weeks for determining *I suis* status at the litter level.

Many studies have shown that *I suis* infection can cause reduced weaning weights,[2,3,7-9,11,20,21] but only a few studies have looked at the impact of isosporosis on postweaning performance.[7,22,23] Study Two clearly showed that some pigs shed oocysts at the time of weaning and for a few weeks afterwards, and that pigs from infected litters were significantly lighter in weight at 8 weeks of age than pigs from non-infected litters. Furthermore, an association between *I suis* infection and the occurrence of watery diarrhea was noted in 3-week-old pigs. The reason for examining the association of pigs from a non-infected or infected litter with the "watery diarrhea" variable was that watery diarrhea is easier to define correctly than pasty feces when more than one person observes and categorizes the feces. It should be noted that coccidiosis is commonly associated with pasty feces, but, in our opinion, classifying slightly abnormal

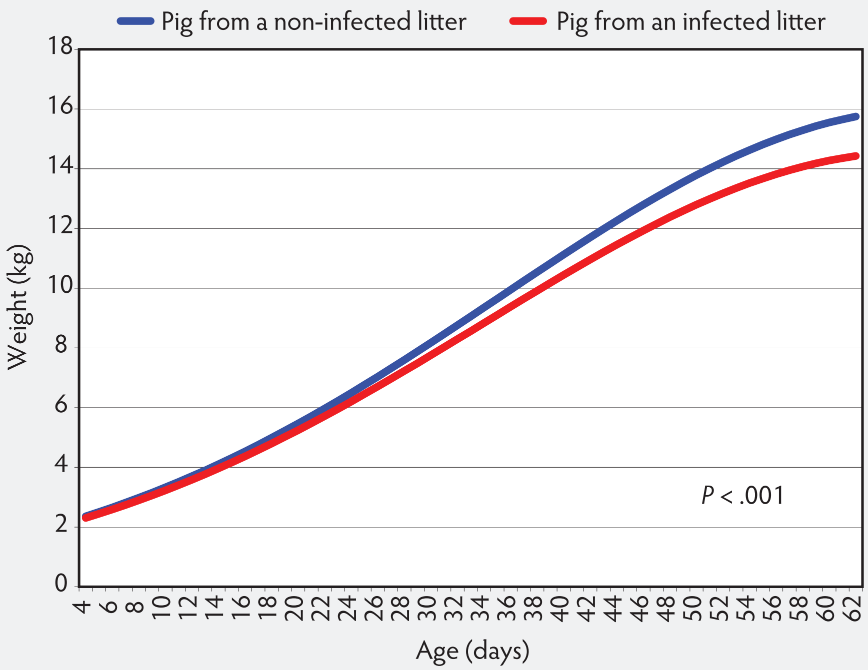
Table 2: Fixed and random effects in a mixed model showing the association between individual pig weight and *Isospora suis* infection at litter level for pigs ≤ 8 weeks of age on three Ontario farms (Study Two)†

Fixed effects					
Parameters	β	SE	*P*	95% CI	
Infected litter	-0.019	0.0421	.65	-0.102	0.063
Age	0.064	0.0006	< .001	0.063	0.065
Age square	-0.0005	8.45 e-6	< .001	-0.0005	-0.0004
Infected litter*age	-0.001	0.0003	< .001	-0.002	-0.0005
Farm F3	0.15	0.056	< .01	0.042	0.262
Farm F4	0.06	0.058	.33	-0.576	0.171
Constant	0.61	0.052	< .001	0.511	0.713

Random effects				
Parameters	s	SE	95% CI	
Litter ID	0.1365	0.0149	0.1103	0.1691
Pig ID	0.2043	0.0065	0.1920	0.2174
s (residual)	0.1337	0.0017	0.1304	0.1370

† Final mixed model constructed on the basis of six weight measurements in 678 pigs 1 to 8 weeks of age from 72 litters on three farms, F2, F3, and F4. Farm F2 was used as the referent farm and is therefore not included in the model.

Figure 2: Study Two: Predicted weight (kg) of pigs over time for *Isospora suis*-infected and non-infected litters as estimated by a linear mixed model. The final model was constructed on the basis of six weight measurements in 678 pigs 1 to 8 weeks of age from 72 litters on three farms.



consistency was too subjective to use in the analysis. The prevalence of oocysts and the association with diarrhea in the present study are similar to the findings of another researcher.[24] *Isospora suis* has been reported as the most common pathogen identified in fecal samples of diarrheic piglets 5 to 30 days of age; in one report, *I suis* oocysts were present in 53.8% of the samples from diarrheic piglets, while enterotoxigenic *Escherichia coli* was present in 18.2% of samples, and rotavirus was found in 16.9% of samples.[25]

In all likelihood, the pigs affected by *I suis* had damage to the intestinal villi and were less able to absorb nutrients than unaffected pigs, with resulting diarrhea[3,7-9,11,20,21,25] and poor growth rates. Although it was beyond the scope of this study to measure feed intake and feed conversion, one can speculate that the damaged gut associated with *I suis* infection is likely to cause a reduction in feed efficiency. However, it is possible that the lighter weight at 8 weeks of age could be a result of lower feed intake. Either way, the economic implications of the findings of this study are important.

Firstly, a difference in weight gain in the nursery may be amplified by the time the pig reaches market weight. Mahan and Lepine[26] showed that pigs weighing < 5 kg at weaning, even when fed a high-quality starter ration, remained smaller than pigs with heavier weaning weights. In their trial, a difference of 1 or 2 kg in the early nursery phase translated to more than 2 weeks longer in days-to-market. Secondly, because nursery feed is very expensive, if feed efficiency is hampered due to poor absorption of nutrients, the cost of the disease is compounded. On many farms, postweaning enteric diseases are major concerns and are a reason for the almost ubiquitous presence of antimicrobials in starter rations. It is possible that if isosporosis were controlled, leading to a healthier intestinal mucosa at the time of weaning, then piglets might be better able to resist infections with enterotoxigenic *E coli* and other gut pathogens.

It is somewhat difficult to compare the results of various *I suis* studies, because researchers often use different methods to determine if a litter of pigs is infected. In the current study, in which three pigs were randomly selected and assessed individually, the chance of detecting an *I suis*-positive litter was increased by examining fecal samples from individual piglets rather than a pooled sample. Dilution associated with pooling samples can mask the fact that one piglet is shedding low numbers of oocysts. Another issue that was at least partially addressed by the sampling design of the current study is the concern that oocyst shedding tends to be intermittent.[9,27] By returning to the same farm and repeating the sampling from the same litters and the same piglets at weekly intervals (2 and 3 weeks of age), rather than sampling pigs on a single occasion, it was possible to increase the likelihood of identifying infected piglets. This is in agreement with Meyer et al,[28] who demonstrated that repeated fecal sampling increases the chance of finding *I suis* oocysts.

In the present study, repeated measures of weights for a single pig were highly correlated, indicated by a high ICC at the pig level. The weights of pigs within a litter were also correlated with one another relative to the total variation (pig level plus litter level plus residual). Thus, it was important to take clustering into consideration when analyzing the association between *I suis* infection and weight gain.

In modeling the relationship between growth and the presence of *I suis* oocysts, it was important not to bias the results by including diarrhea in the model, because other agents besides *I suis* might be responsible for causing diarrhea and affecting weight gain. There was also a concern that the observation of diarrhea at the time of sample collection alone might be inadequate to truly characterize the extent and severity of the condition. In future studies, the relationship between *I suis* oocyst shedding and postweaning diarrhea might be examined more closely by daily clinical scoring of the consistency of piglet feces, as reported previously,[29] and by closely examining the interaction of coccidia and other enteric pathogens. In addition, further studies might utilize alternative diagnostic tools, such as qualitative detection of oocysts by autofluorescence,[30] in order to increase the accuracy of the study.

It must be emphasized that the farms enrolled in this study were selected because of a history of isosporosis and are not necessarily representative of all Ontario pig farms. The prevalence and impact of coccidia may be different on other farms.

Implications

- *Isospora suis* infection can have an important impact on the performance of pigs in the early postweaning period.

- Farmers and veterinarians should reassess their coccidiosis control programs on the basis of prevalence and severity of the disease in newly weaned pigs, in addition to suckling piglet performance.

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