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Original Article

Cystoisospora suis infection in suckling piglets in Brazil: Prevalence and associated factors

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A R T I C L E I N F O

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A B S T R A C T

Piglet coccidiosis is a parasitic disease caused by the protozoan *Cystoisospora suis,* which is regarded as the most prevalent gastrointestinal parasite in intensive pig farms. Despite the availability of highly effective chemo-metaphylaxis (toltrazuril), coccidiosis is still prevalent in European and other countries. We conducted a cross-sectional study on swine farms in Brazil, to assess the prevalence of *Cystoisospora suis* in fecal samples and determined the associated factors. In total, 666 litters from 50 farms were sampled twice within one week between samplings (mean age at sampling: 10.75 and 17.7 days). Of 666 litters, 225 (33.8%) were positive at least once, and the expected within farm prevalence of *C. suis* oocysts was 32.9% (25.4–41.3%; 95% confidence interval). Oocysts were more prevalent in fecal samples collected from farms with diarrhea (odds ratio = 6.75). The room temperature was also positively associated with oocyst detection; a one-degree increase in room temperature increased the chance of a litter being positive by 23.2%. Up-to-date, this is the most comprehensive technical evidence of factors associated with *C. suis* infection in Brazilian industrial piglet farms.

1. Introduction

Piglet coccidiosis is a parasitic disease caused by the protozoan *Cystoisospora suis,* which is regarded as the most prevalent gastrointestinal parasite in intensive pig farms (Lindsay et al., 2019). Although diarrhea represents the most common manifestation of this disease, subclinical cases have been observed frequently. In both situations, the parasite damages the intestinal mucosa, leading to impaired intestinal function and decreased production parameters, such as average daily gain and feed conversion. Weight reduction may reach 1000 g at weaning, and this negative effect is also observed in sub-clinically infected animals (Joachim et al., 2019; Maes et al., 2007). *C. suis* can also interact with other enteropathogens such as rotavirus, transmissible gastroenteritis virus, clostridia, and *Escherichia coli,* leading to increased clinical infection and mortality (Stuart and Lindsay, 1986; Joachim and Shrestha, 2019).

The life cycle of *Cystoisospora suis* involves an exogenous and endogenous phase. Infected piglets shed oocysts through feces and, under certain environmental conditions (temperature, humidity, and oxygen), oocysts sporulate and become infectious. The route of infection is fecal-oral, and oocysts are quickly transmitted between litters in the corresponding batches. Transmission is favored by the high resistance of oocysts in the environment and the short endogenous phase, with patency of four to seven days, leading to high and permanent exposure of neonatal piglets (Joachim and Shrestha, 2019).

The control of parasite infection is based on the timely interruption of the parasite's development cycle in the gut, preventing intestinal damage, development of clinical signs, and production of oocysts. Disinfection is an important preventive measure against many intestinal pathogens in piglet farms; however, when considered alone, it has a limited effect on coccidiosis control as oocysts are resistant to commonly used disinfectants (Harleman and Meyer, 1983; Hinney et al., 2020). Despite the availability of highly effective chemo-metaphylaxis (oral toltrazuril), coccidiosis is still prevalent in important swine producing countries worldwide (Chan et al., 2013; Gong et al., 2021; Ruiz et al., 2016).

Hence, a better understanding of the environmental and management factors that drive *C. suis* infection in piglet farms is required. Such epidemiological studies were conducted in European countries to improve the control of this parasite (Hinney et al., 2020). Despite being an important pig producer (the 4th largest exporter of pork and 4th largest producer worldwide) (ABPA, 2020), there is no up-to-date

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information on the prevalence and factors associated with piglet *C. suis* infection in Brazil. Previously published case-control studies have focused mainly on the association of diarrhea with the detection of *C. suis* and other pathogens involved in neonatal/pre-weaning diarrhea, covering a limited geographical area regarding the national production chain (Lippke et al., 2011; Ruiz et al., 2016).

This study aimed to assess the prevalence of *C. suis* infection in suckling piglets in commercial piglet farms in Brazil. We further describe factors associated with *C. suis* infection in piglets, such as health parameters (diarrhea), management characteristics, disease control measures, and farm characteristics.

2. Material and methods

This cross-sectional field study was designed to assess the prevalence of *Cystoisospora suis* infection in intensive Brazilian pig farms. Fifty-one farms were selected from January to October 2021 by convenience according to the farmer's willingness to participate. Eight Brazilian states, where >95% of the pig/pork production is concentrated, were included in the study (States of Santa Catarina (18 farms), Rio Grande do Sul (9), Paraná (7), São Paulo (2), Minas Gerais (9), Mato Grosso (3), Mato Grosso do Sul (1), and Goiás (2)) (Embrapa, 2020). According to the climate classification of Kottek et al. (2006), 70.6% of the sampled farms are located in 'Warm temperate climate, fully humid', characterized by high humidity and wide temperature range throughout the year. The remaining 29.4% are located in 'Equatorial savannah with dry winter' climate, characterized by high temperatures all year, but humid summers and dry winters.

This sample size is large enough to estimate a within-farms prevalence of *C. suis* of 5% (Sartor et al., 2007), with a 95% confidence level and a relative margin error of 6%. Ten litters per batch (or 10% of the litters for farms with >2000 sows) were selected and sampled per farm. The sampled litters were selected by convenience sampling and proportionally to the parity order distribution of the corresponding farrowing batch.

Fecal samples were collected from a minimum of five different points within the farrowing crate before the first-morning inspection of the farrowing house, anticipating that piglets have a natural tendency to use separate locations for elimination with high frequency at a young age (Fraser and Broom, 1997). Samples were pooled per litter (minimum of five piglets and total of 50–100 g per fecal pool) and analyzed using the flotation method. Flotation is the routine and accredited technique applied in the two laboratories involved in this study (Supplementary material I). To increase sensitivity, each litter was sampled twice with a one-week interval between samplings (mean age at sampling: 10.75 and 17.7 days), as previously described (Joachim et al., 2018a; Hinney et al., 2021). A litter was considered coccidian-positive when at least one of the two samplings was positive, and no distinction was made for litters that tested positive once or twice. A farm was considered positive when at least one pool of litter was positive, as previously defined (Hinney et al., 2021).

In each participating farm, a questionnaire was provided to collect information on the farm structure, management, and medication of piglets during the suckling period, as well as previous records of diarrhea (Table 1). This questionnaire was completed by a farm veterinarian.

Table 1
Description of the variables obtained through the questionnaire in all 51 studied farms in Brazil.

Diarrhea: dichotomic variable describing presence *vs* absence of diarrhea in the room where the sample was taken.
Aclimatization system: dichotomic variable describing the presence of an automated system for temperature and air quality control (air-conditioning) *vs* temperature and air control based on the natural ventilation system.
All-in all-out: categorical variable describing if the farm is applying all-in/all-out system *vs* continuous system.
Antimicrobial treatment: dichotomic variable for the use of antimicrobials as a prophylactic measure for disease control *vs* no preventive use but curative.
Farrowing assistance: dichotomic variable for the assistance during the farrowing, it could be totally assisted (from the start until the end of the farrowing) *vs* partially assisted (only during 'working session').
Management system: categorical variable regarding the piglet batch management (one week *vs* more week batching).
Floor type: categorical variable describing farrowing crate floor type: a solid concrete floor, *vs* slatted concrete floor.
Feed day: quantitative variable for the age at which piglets started receiving pre-started solid feed.
Number of sows (sows/farm): quantitative variable for the number of sows in the farm.
Piglets/sow: continuous variable to describe the average number of live piglets born per sow (obtained in corresponding batch).
Room temperature: continuous variable for the temperature (°C) of the farrowing room where the sample is collected (obtained at the middle corridor of farm).
Parity order: ordinal scale for the number of farrowings of a sow.
Coccidiosis control protocol: categorical variable describing if the piglets were treated with one *vs* two applications of toltrazuril *per-os*.
Disinfection protocol: dichotomic variable indicating if the disinfectants used in the room are effective in inactivating the parasite. The effectiveness was claimed by the manufacturer.

2.1. Statistical analysis

A descriptive statistical analysis was used to explore the characteristics of the study population. To assess the factors associated with the presence or absence of oocysts in each litter, a generalized linear mixed model (McCulloch and Searle, 2001) was applied using the logit link function and a 'residual' Bernoulli distribution. The linear predictor of the model included fixed effects for the parity order, piglets/sow, room temperature, feed day, sows/farm, coccidiosis control protocol, presence of diarrhea, acclimatization system, all-in all-out system, use of antimicrobial treatment, farrowing assistance, management system, floor type, and disinfection protocol. The farm was used as a random effect in the model. Candidate variables for the multivariable model were screened using univariable analysis. Variables with a *p*-value of <0.25 were selected for inclusion in the multivariable model. Stepwise procedures were used to select statistically significant variables for the final multivariable model. At each step, variables with the smallest p-value were included until the Akaike criteria information (AIC) stopped decreasing. Variables used in the model were removed if their p-value exceeded the significance level of 0.05 until only significant variables remained in the model. It was assumed that the variables number of sows and parity order represent the herd size and age of the sow, respectively, and were tested in the final model as confounders by the observation of variations (20% or higher) in the estimates of significant variables (Serafini Poeta Silva et al., 2019).

Random effects were assumed independent and normally distributed around zero, with the respective variance components. Approximate maximum likelihood inference was based on the Laplacian integration, as implemented in R (R Core Team, 2019) in routine glmmTMB from the glmmTMB library (Brooks et al., 2017). Fixed effects were tested with the classic Wald test, employing an approximation by the chi-square distribution, as implemented in the R routine Anova from the car library (Fox and Weisberg, 2019).

3. Results

3.1. Characteristics of the study population

3.1.1. Management

Fig. 1 shows the overall descriptive statistics for the management characteristics in farms. Regular iron application for the piglets was reported in all farms, and three out of 51 (5.9%) did not use metaphylactic treatment of coccidiosis. The average age of piglets at toltrazuril application was three days, standard deviation (SD) of 0.32 days. Most of the farms (76.4%) used an oral product as a single application according to SPC; 17.6% of farms used medication twice during the first

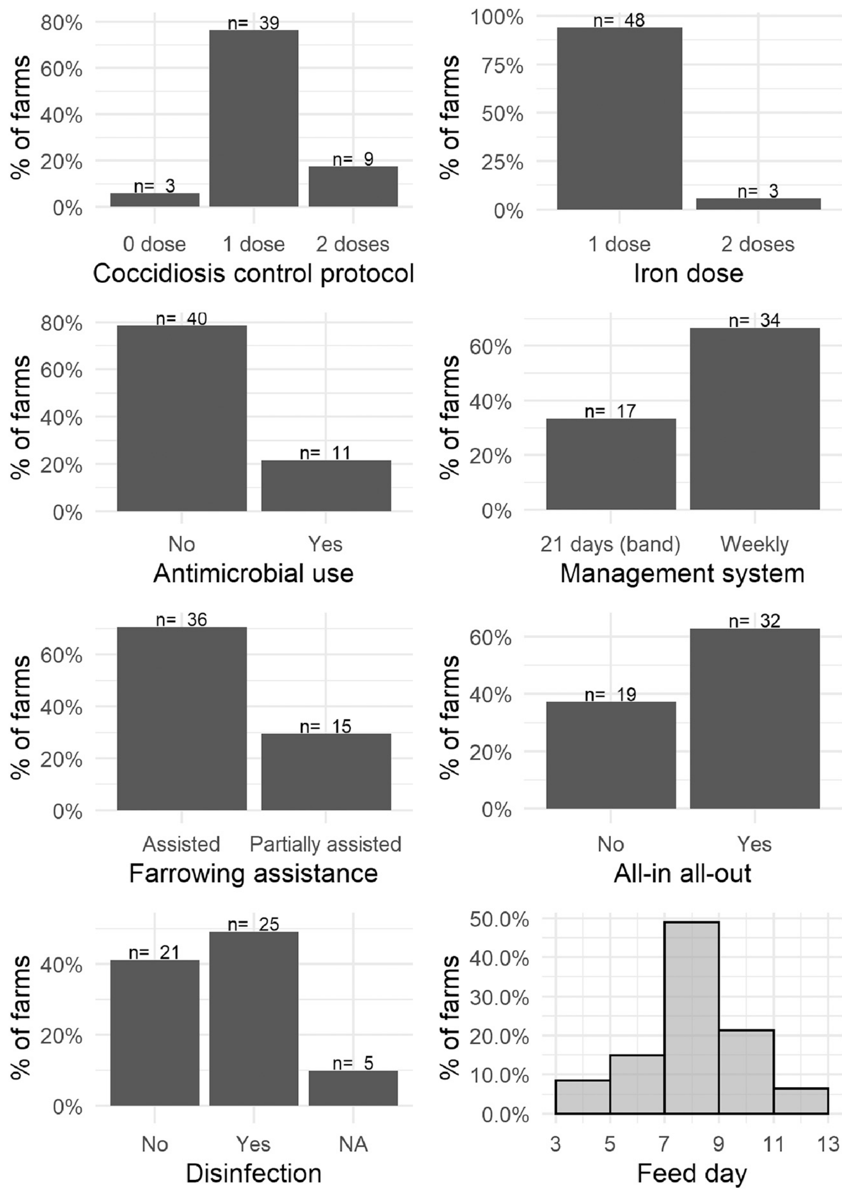

Fig. 1. Description of the study population regarding management characteristics for the 51 farms included in the survey, including the number (n) and percentage of farms per category and variable. For feed day, the histogram represents the distribution of the feed day over the 51 studied farms. NA = not available data.

week of piglets' age. Forty farms (78.4%) did not use routine antimicrobials as the prophylactic measures during the preweaning period. The dominant management system was one week farrowing (66.7%), and the remaining 33.3% in 21 days intervals. Farrowing assistance was reported in 70.6% of the farms, and the remaining 29.4% used partial farrowing assistance (during the day hours). Most farms (62.7%) used multi-site systems (2–3 site management), and 37.2% used farrow-to-finish farm organization. Approximately 9.8% of 51 farms did not provide specific information about cleaning and disinfection on a regular basis. Twenty-five (49%) farms used commercial disinfectant products with registered efficacy claim against coccidiosis. Twenty-one (41.17%) did not use disinfectant products with registered efficacy claim against coccidiosis. The majority of the farms used disinfection based on a combination of quaternary ammonium, glutaraldehyde, ethanolic aldehyde, and chemical enhancers. The average age (days) for solid feed distributed to piglets was 7.9 (±2.5, SD).

3.1.2. Structure of farms

Fig. 2 shows the descriptive statistics of the farm structure/herd characteristics. The automated climatization system was used in 54.9% of the farms, while 45.1% of farms used natural ventilation. Concrete slat was the main floor type (74.5%) in farrowing houses; 19.6% used a mixture of concrete slats and compact concrete floors, and only 5.9% used complete compact concrete floors. Diarrhea was reported in 58.8% of the farms. The mean room temperature was 23.8 °C (±3.7, SD); the average number of live-borne piglets per sow was 13.4 (±1, SD); and the average number of sows per farm was 1800 (±1.700, SD), with a mean parity order of 3.4 (±2.11, SD).

3.2. Prevalence of C. suis infection and associated factors

In total, 666 litters were sampled from the 50 farms for the prevalence of *C. suis* (one farm was excluded because of missing information). In total, 225 litters (33.8%) were positive at least in one of the samples, and the expected *C.suis* within farm prevalence fit by the logistic regression model was 32.9%, 25.4–41.3%, 95% confidence interval (95% CI). Four categorical variables (diarrhea, all-in all-out, farrowing assistance, floor type), with $p < 0.25$ in the univariable analysis, were

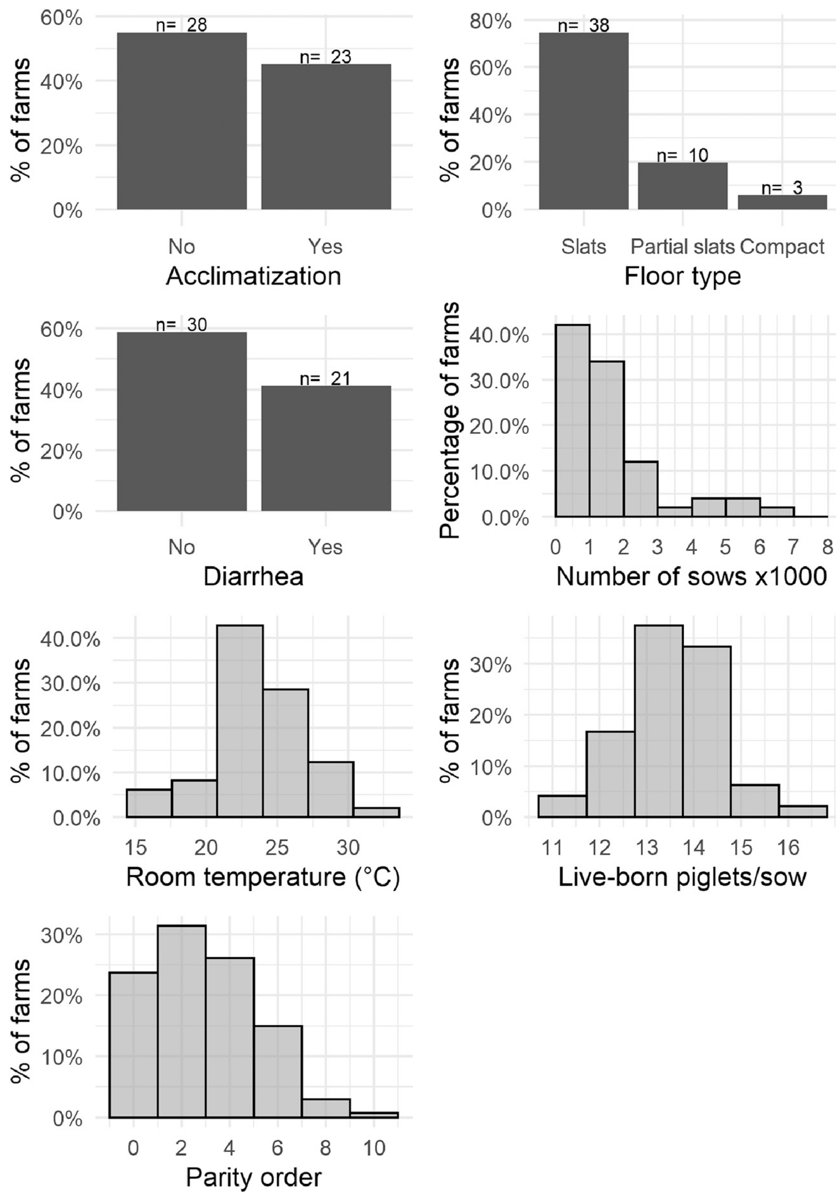

Fig. 2. Description of the study population regarding farm structure/characteristics of the herd for the 51 farms included in the survey, including the number (n) and percentage of farms per category and variable. Histograms describe the distribution of each variable over the 51 studied farms. The variable Parity order is observed on litter level.

selected for the multivariable model (Table 2). Further, room temperature and piglets/sow, with *p*-value <0.25, were also selected for the multivariable model (Fig. 3). For the final model, the stepwise selection retained diarrhea and room temperature, with an of AIC 698.3 (summary for the stepwise selection is available in Supplementary material II), and no confounding effects of the number of sows and parity order were observed. The variance component for the farm was 1.075, and the intracluster correlation indicated that the farm explained 24.6% of the variation in the prevalence of oocysts. On average, farms with diarrhea had a higher chance (odds ratio = 6.75) of being positive for *C. suis* than that farms without diarrhea (Table 3). The room temperature was also positively associated with the occurrence of oocysts; a one-degree increase in the room temperature increased the chance of a litter being positive for oocysts by 23.2% (Table 3 and Fig. 4).

4. Discussion

This cross-sectional study aimed to assess the prevalence of *C. suis* and associated factors in commercial (industrial-scale) piglet-producing farms in Brazil. Previous studies were not designed to estimate prevalence but were mainly based on case-control designs (Lippke et al., 2011; Ruiz et al., 2016) or focused on a limited target population (Sayd and Kawazoe, 1996). In the present study, we performed samplings in the regions with the highest pig production, representing approximately 95% of Brazilian pig production (Embrapa, 2020), allowing us to provide a robust estimative for the *C. suis* infection prevalence in suckling piglets in Brazilian commercial farms.

The expected mean within-farm *C. suis* infection prevalence reported in this study (32.9%) is lower than the approximately 50% within-farm prevalence observed in Austria, the Czech Republic, Germany, and Spain by Hinney et al. (2020) using samples taken in the 2nd and 3rd week of piglets' life. It is important to remember that Hinney et al. (2020) used the autofluorescence method to detect *C. suis* oocysts in feces. The flotation is less sensitive than autofluorescence (Joachim et al., 2018a), which could explain why the prevalence observed in our survey is lower. However, this limitation does not hamper the associated factors because

Table 2
Frequency of litters (n = 666) in each variable category and the distribution of *C. suis* positive litters. Results for the univariable analysis were obtained by the classic Wald test, p-values <0.25 considered for the multivariable model.

Variables (p-value)	*N* observations (%)	% oocyst-positive samples
Coccidiosis control protocol (0.96)		
No dose	40 (6.00)	2.00%
One dose	512 (82.98)	35.15
Two doses	105 (17.02)	41.90
Diarrhea (<0.001)		
Yes	291 (43.69)	46.69
No	375 (56.31)	21.33
Acclimatization system (0.6)		
Yes	340 (51.06)	33.23
No	326 (48.94)	34.35
All in all out (0.0112)		
Yes	443 (66.51)	28.44
No	223 (33.48)	44.39
Antimicrobial treatment (0.84)		
Yes	140 (21.02)	35.00
No	526 (78.98)	33.46
Farrowing assistance (0.0077)		
Completely	486 (72.97)	29.01
Partially	180 (27.03)	46.66
Management system (0.35)		
Band	231 (34.68)	29.43
Weekly	435 (65.32)	36.10
Floor type (0.02)		
Concrete slats	494 (74.17)	30.36
Partial concrete slats	141 (21.17)	48.22
Compact	31 (4.66)	22.50
Disinfection protocol (0.45)		
Yes	321 (48.20)	39.25
No	305 (45.80)	27.86
Missing	40 (6.00)	35.00

the same diagnostic test is used in all samples, making all levels of all covariables comparable.

The ability of *C. suis* to cause diarrhea in infected piglets has been well documented in experimental and field studies (Harleman and Meyer, 1983; Joachim et al., 2018b). Pasty feces (fecal score of 2) correlated significantly with the presence of oocysts (Hinney et al., 2020). In our case, a detailed evaluation of the fecal score of the pool samples was not performed; thus, the correlation between the presence of oocysts and fecal consistency scores was missing. The variable diarrhea does not 'explain' the *C. suis* infection, but it is strongly associated with the outcome because it is the main clinical manifestation of infection (Joachim et al., 2018b). Thus, diarrhea is a consequence of infection and subsequent alteration of the small intestinal mucosa, and our model captured this association.

Infection of piglets by coccidia occurs in the fecal-oral cycle. Like most other endoparasites, *C. suis* life cycle depends on favorable environmental conditions. The positive correlation between room temperature and the occurrence of *C. suis* can be explained by the fact that the life cycle of coccidia species, such as *C. suis,* is dependent on an extra-host step involving the sporulation of the oocyst into an infective sporulated oocyst (Harleman and Meyer, 1983). Higher temperatures accelerate the sporulation of oocysts in the environment (Graat et al., 1994) or increase the sporulation rate (Langkjær and Roepstorff, 2008), leading to higher exposure levels. In the present study, the room temperature was positively associated with *C. suis* infection (oocyst positivity). As previously described by Lindsay et al. (2019), supplemental heat in the range of 32–35 °C provided to newborn piglets promotes rapid development of oocysts in farrowing crates. In contrast, the combination of temperatures (25–30 °C) and low relative humidity levels (53–62%) had a negative effect on oocysts (Langkjær and Roepstorff, 2008). However, in Brazil, the humidity is generally higher than 62% during the whole year. In the south region, where 70.6% of the samples were obtained, the relative humidity ranges between 76% and 88% during the year (INMET, 2022; USP, 2022) and may contribute to the optimal environment for the external cycle of parasite sporulation (Wathes and Whittemore, 2006).

No other variables had a significant effect on the presence of oocysts. However, previous studies have reported improvements in pigs' health in the all-in, all-out production system during different stages (Sperling et al., 2020). Segregated rearing using the all-in, all-out system could reduce the risk of piglets becoming infected by ingesting sporulated oocysts in contaminated farrowing pens (Pettersson et al., 2019). Also, no significant effect of the disinfection using disinfectants with a claim against parasites was observed on the oocyst presence. Cleaning and disinfection of farrowing pens need to be carried out effectively; in particular, disinfectants should be effective against parasites. In our study, no differences were observed in efficacy between the products with and without a specific *C. suis* claim efficacy. Previous reports suggest that a limited number of farms were using disinfectants effective against oocysts according to their summary of product characteristics (Hinney et al., 2020). Besides the product characteristic, several physical and chemical factors also influence the efficacy of disinfectant procedures: concentration, temperature, pH, relative humidity, and water hardness, together with the period of mechanical cleaning and drying. Mistakes in such practices may explain the absence of appreciable differences between farms applying disinfection and those, that did not.

Despite the high treatment rate observed in this study in comparison to previous reports (Hinney et al., 2020), the efficacy of treatment based on oocyst shedding was low, and no difference was observed in oocyst shedding between the farms applying single or two doses of toltrazuril during the first week ($p = 0.96$). The endogenous phase of the parasite is limited to the first five days after infection (with the first piglets becoming infected just after birth), and the "window of opportunity" for intervention against endogenous stages is very small. Toltrazuril and its active metabolite toltrazuril sulfone have prolonged effective drug concentration at the target site of infection and long halftime of elimination. Consequently, a second application will not increase the efficacy of anticoccidial drugs (Karembe et al., 2021). Toltrazuril is considered highly efficient in the control of experimentally induced cystoisosporosis, with limited evidence of resistance (Joachim and Mundt, 2011; Kreiner et al., 2011; Maes et al., 2007; Mundt et al., 2007; Rypula et al., 2012; Shrestha et al., 2017). However, there are reports that oocyst shedding can occur despite toltrazuril treatment, and its complete suppression by treatment is challenging to achieve, particularly under field conditions (Hiob et al., 2019; Kreiner et al., 2011; Mundt et al., 2007; Mundt et al., 2006).

The main reasons for the lack of efficacy may be application errors (*e.g.* vomiting or regurgitation after oral application), too late treatment in relation to infection and insufficient hygiene practices. Our aim here was not to detect the reasons for the treatment failure, but our results highlight the need for further investigation of the lack of effectiveness in field conditions.

A limitation of our study is that the sampling strategy did not account for stratification per state of the country, and the scarcity of farms sampled for some individual Brazilian states did not allow us to compare between-state infection prevalence. Furthermore, convenience sampling instead of random sampling may have led to a biased sample. Selection bias is likely present in non-random sample-based studies, but real random sampling in epidemiological studies is complex because farmers must be willing to participate. Rothman et al. (2013) argued that in such situations, the results of the associated factors remain valid, helping to seed hypotheses and evaluate efficacy of treatment in the field. This study provides the most up-to-date evidence regarding the prevalence of *C. suis* and associated factors in intensive piglet production in Brazil.

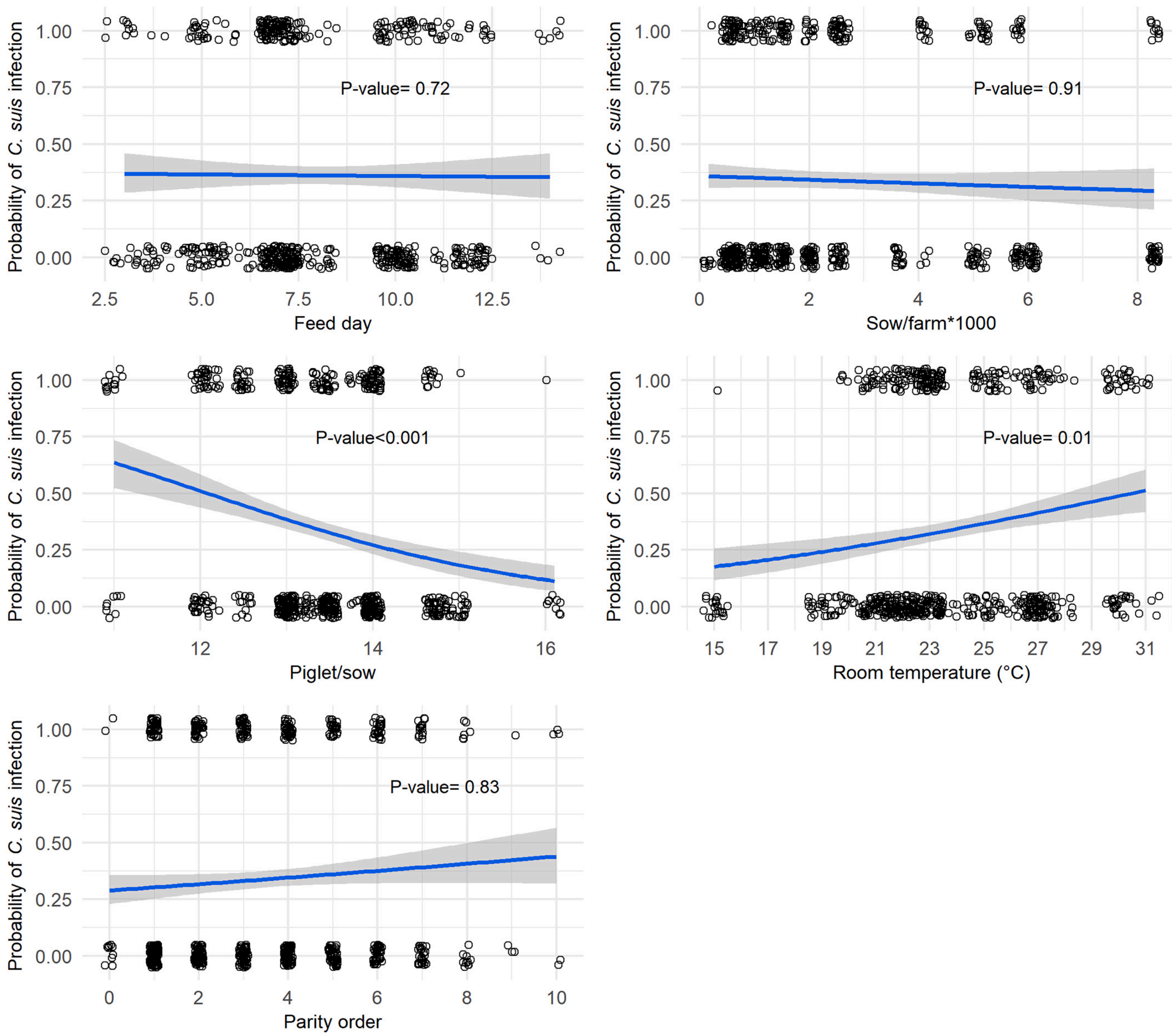

Fig. 3. Results for the univariable analysis for the five quantitative variables. Variables representing *p*-values <0.25 were considered for the multivariable model.

Table 3
Summary of the final multivariable model to predict the prevalence and associated factors of *C. suis* infection in suckling piglets in Brazilian commercial farms.

Variable	Estimate	Odds ratio (95% CI)*	Prevalence**	p-value
Diarrhea				<0.001
Yes	1.91	6.75 (3.18–15.5)	53.8%	
No	–	1	14.71%	
Room temperature	0.208	1.23 (1.09–1.39)	–	<0.001

* CI = confidence interval.
** Predicted prevalence.

5. Conclusion

We observed an association of *C. suis* with diarrhea and a correlation of parasite prevalence with room temperature. This is the most recent and comprehensive study on *C. suis* and associated factors in Brazil.

Ethics approval and consent to participate

In this study, fresh fecal samples from living animals were collected from the floor during farm visits by attending veterinarians, and examination was part of the standard veterinary practice procedure applied on farms, so that no animals were directly involved. No conditions or interventions were changed for the included farms. Owing to the characteristics of the study without manipulation of animals, ethical approval was not necessary. The farmers consented to participate in the questionnaire.

Funding

This research received no external funding.

Data availability statement

All data are included in this publication.

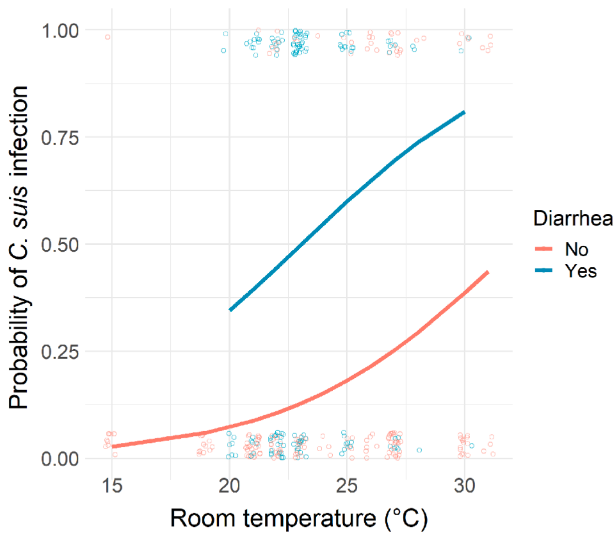

Fig. 4. Predicted probability of *C. suis* infection according to the room temperature (°C) in litters with and without diarrhea. The solid lines and dots indicate predicted probability and observed data, respectively.

Declaration of Competing Interest

DS, HK, and JC are employees of Ceva. EFC is an employee of Wageningen Bioveterinary Research Institute, The Netherlands.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2022.100796.

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