Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2022v43n4p1695

Evaluation of different transmission routes of *Cystoisospora suis* in a farm using prophylactic toltrazuril

Avaliação de diferentes vias de transmissão de *Cystoisospora suis* em uma granja utilizando toltrazuril profilático

Arielle Aparecida Lara[1]; Ricardo Babinski Bregonde[2]; Ana Paula Molinari Candeias[3]; Vinicius Dahm[4]; Geraldo Camilo Alberton[5]; Alexandre Leseur dos Santos[5]; Silvia Cristina Osaki[5]*

Highlights

C. suis is not widely disseminated within the studied maternity environment.
Water present in the drinking fountains could be the mainly route of transmission.
Treatment based on toltrazuril did not interfere with the occurrence of protozoa.

Abstract

Coccidiosis is an enteric disease caused by protozoa, especially *Cystoisospora suis* which can lead to large losses in production. Its transmission occurs through different routes, mainly affecting piglets in the first weeks of life and normally progressing with diarrhea unresponsive to antibiotic therapy. The objective of this work was to evaluate, in two seasons of the year, the occurrence of C. suis in piglets and sows, the transmission routes and source of infection and the influence of the preventive use of toltrazuril on these epidemiological parameters. Two experiments were carried out, the first being in winter; under the influence of prophylactic use of toltrazuril; and the second in the summer, six months after the suspension of the prophylactic use of toltrazuril. The study included 36 sows (18 controls, 18 treated toltrazuril). The following samples were collected: rest of the sows, water from the individual drinkers in each cage, organic swabs present in the keepers' shoes, environmental swabs pre and housing, present in the cages in the

[1] Student of the Master's or Doctoral Course of the Postgraduate Program in Animal Science, Universidade Federal do Paraná, UFPR, Palotina Sector, Palotina, PR, Brasil. E-mail: ariellelara91@gmail.com
[2] Undergraduate Student in Veterinary Medicine, UFPR, Palotina Sector, Palotina, PR, Brasil. E-mail: ricardobabinskib@gmail.com
[3] Student of the Master's or Doctoral Course of the Postgraduate Program in Animal Science, UFPR, Palotina Sector, Palotina, PR, Brasil. E-mail: anapmolinaric@gmail.com
[4] Undergraduate Student in Veterinary Medicine, UFPR, Palotina Sector, Palotina, PR, Brasil. E-mail: vinicius_dahm@live.com
[5] Profs. Drs., Course of the Postgraduate Program in Animal Science, UFPR, Palotina Sector, Palotina, PR, Brasil. E-mail: alberton@ufpr.br; alsantos@ufpr.br; sil_osaki@ufpr.br
* Author for correspondence

Received: Sept. 01, 2021 - Approved: May 03, 2022

rooms and accumulations of the maternity cage. As were disclosed to coproparaological and PCR exams. The presence of C. bebe ocysts is the winter and summer control following samples: sow feces (2.84%) of the winter and summer control groups (7.60%) of the winter and summer treated groups and the treated group winter and summer, rectal swabs from litters (9.03%) in the control and treated groups in winter, collected from the environment (20.22%) from all groups. Like others, they were negative for the presence of the parasite. There was no difference between the control and toltrazuril groups and no positive samples were observed in the summer. The possible route of transmission of the agent in the present work was the water from the douros. Preventive therapy with toltrazuril in farms with low infection pressure is not necessary to control *C. suis.*
Key words: Anticoccidial. Coccidiosis. Pig.

Resumo

A coccidiose é uma doença entérica, causada por protozoários, destacando o *Cystoisospora suis* que pode levar a grandes perdas na produção. Sua transmissão ocorre por diferentes rotas, afetando principalmente leitões nas primeiras semanas de vida e cursando normalmente com quadros de diarreia não responsivos à antibioticoterapia. O objetivo do trabalho foi avaliar, em duas estações do ano, a ocorrência de *C. suis* em leitões e matrizes, as vias de transmissão e fonte de infecção e a influência do uso preventivo do toltrazuril sobre estes parâmetros epidemiológicos, Dois experimentos foram realizados, sendo o primeiro no inverno; sob efeito do uso profilático do toltrazuril; e o segundo no verão, seis meses após a suspensão do uso profilático do toltrazuril. O estudo incluiu 36 porcas (18 controles, 18 tratadas com toltrazuril). As seguintes amostras foram coletadas: fezes das porcas, água dos bebedouros individuais de cada gaiola, suabes retais de cada leitegada, resíduos orgânicos presentes no calçado dos tratadores, suabes ambientais pré e pós alojamento, insetos presentes nas salas analisadas e fezes acumuladas nas gaiolas da maternidade. As amostras foram submetidas a exames coproparasitológicos e PCR. A presença de oocistos de *C. suis* foi detectada nas seguintes amostras: fezes das porcas (2,84%) dos grupos controle do inverno e verão, água dos bebedouros (7,60%) dos grupos tratado do inverno e verão e do grupo controle do verão, suabes retais das leitegadas (9,03%) nos grupos controle e tratado no inverno, fezes coletadas do ambiente (20,22%) de todos os grupos. As outras amostras foram negativas para a presença do parasita. Não houve diferença entre o grupo controle e tratado e não foram observadas amostras positivas no verão. A possível via de transmissão do agente no presente trabalho foi a água dos bebedouros. A terapia preventiva com toltrazuril em granjas com baixa pressão de infecção não é necessária para o controle de *C. suis.*
Palavras-chave: Anticoccidiano. Coccidiose. Porco.

Introduction

Coccidiosis in litters is an enteric disease caused by Coccidia class protozoa, with the species *Cystoisospora suis* being the main species related to production losses (Mundt et al., 2006). Few studies, mainly those related to the frequency and epidemiology of gastrointestinal parasites, have been carried out to investigate swine helminths and coccidia infections in Brazil (D'Alencar et al., 2006). Some studies carried out in Brazil demonstrate the occurrence of C. suis in several states, such as: São Paulo: 13.7%

(Nishi et al., 2000); 10.9% (Calderaro et al., 2001) and 34.78% (Ruiz et al., 2016); Santa Catarina: 5% (Sartor et al., 2007); Pernambuco: 0.44% (D'Alencar et al., 2006) and Minas Gerais: 22.8% (Nishi et al., 2000).

Studies that report the effects of management practices, hygiene, sanitary emptiness in the maternity room, and the structure of the farm facilities on the risk of disease occurrence and transmission, are also uncommon (Sotiraki et al., 2008).

Litters can become infected by ingesting sporulated oocysts present in the environment, from contaminated feces remaining from other previous litters, or being carried from other maternity rooms through insects, rodents, and workers, or by contamination on sow ceilings. The oocysts have a high resistance in the environment, being able to survive in the soil for 15 months at a temperature of 40 to 45°C, which could justify the difficulty in controlling and eradicating coccidiosis in the farms, even after the improvements made to cleaning and disinfection practices in current pig farming (Linhares et al., 2012).

The prophylactic use of drugs with anticoccidial action in litters during the first week of life is already routine in most pig farms. Among the active ingredients available for use in these animals, the most efficient is toltrazuril, which when compared with other available drugs, such as diclazuril and sulfonamides, shows superior effects (Mundt et al., 2006).

Toltrazuril is a drug with anticoccidial and antiprotozoal activity, having a wide spectrum of action, and can be used to prevent neonatal coccidiosis in pigs (Adams, 2003). It acts on the different evolutionary forms of the parasite, mainly in schizonts, macrogametocytes, and microgametocytes, changing the function of the respiratory chain and mitochondrial enzymes (Spinosa et al., 2002).

Given the above, the objective of the study was evaluate, in two seasons of the year, the occurrence of Cystoisospora suis in piglets and sows, the transmission routes and source of infection and the influence of the preventive use of toltrazuril on these epidemiological parameters in a pig farm in the municipality of Palotina, Paraná, Brazil.

Material and Methods

Animal Welfare Statement

This study is following the Ethical Principles of Animal Experimentation and was approved by the Ethics Committee on the Use of Animals at the Federal University of Paraná-Setor Palotina, with protocol number 25/2017.

Study location, animals, and management

The experiment was carried out in a commercial farm, in the municipality of Palotina, Paraná, Brazil. The city is in western Paraná, has a humid subtropical climate with hot summers and cold or mild winters, and an annual average temperature of 20°C. The latitude is 24° 17' 2" S and longitude 53° 50' 2"W, having an altitude of 335 m with an average annual rainfall of 1600 to 1800 mm (Sistema Meteorológico do Paraná [SIMEPAR], 2019). The farm was chosen for the study because it had been using toltrazuril for more than 10 years.

A total of four maternity rooms were used, with nine cages in each. The mothers, both multiparous and primiparous, remained in these rooms until the litters were weaned. The 'all-in-all-out' management system was implemented, with just one day's sanitary empty and the environment was washed and disinfected.

The total number of sows used for this experiment was 36 (18 Control group, 18 Toltrazuril group)., belonging to the genetic DB. In maternity, the animals were found in conventional facilities.

Trial Period and experimental design

To understand the epidemiological behavior of *C. suis* with and without the prophylactic use of Toltrazuril, the experiment was carried out using two experimental groups: TOL - Group in which prophylactic administration of toltrazuril occurred (1mL of 5% toltrazuril via the oral route) and the control group (CON), where the animals received 1mL of saline solution orally. The experiment was carried out in two stages: the first in winter and the other in summer. The prophylactic use of toltrazuril as a routine on the farm preceded before and during the winter stage and was suspended until the summer phase.

In the winter and summer stages, half of the sows were included in the TOL group and half in the CON group. The experiment carried out in winter covered the months of July and August 2017, thus covering part of the Brazilian winter, where two maternity rooms were used, nine cages in each one. The experiment carried out in the summer took place during February and March 2018, covering part of the Brazilian summer, with the same number of maternity rooms and cages that were used in the winter.

Sample Collection and Processing

Different biological and environmental samples were collected to verify the possible sources of infection and transmission routes responsible for the transmission of the parasite to the litters (Table 1). The samples were tested by coproparasitological methods (Willis-Mollay and Sheater) and using the polymerase chain reaction (PCR) according to Samarasinghe et al. (2008). DNA extraction for the samples that underwent PCR was performed using a commercial kit DNeasy Blood and Tissue (Qiagen - Hilden, Germany) according to the manufacturer's recommendations, with a final volume of 200 µL. Four freeze/thaw cycles were previously performed to ensure efficient lysis of the wall of all possible oocysts present, before being subjected to the DNA extraction. The amplified samples were analyzed using agarose gel (1,5%) electrophoresis and ethidium bromide staining.

Table 1
Samples collected, days and places of collection and technique used for the detection of *C. suis* oocysts

Sample	Number of samples	Collection days	Collection places	Test
Sow feces	144	-5, 0, 7, 14, 21	Rectal Ampoule	W/S/PCR
Litter feces	144	0, 7, 14, 21	Rectal Ampoule	PCR
Feces in the cage - sow	89	0, 7, 14, 21	Floor	W/S/PCR
Feces in the cage - litter	94	7, 14, 21	Floor	W/S/PCR
Water from drinking fountains	184	-5, 0, 7, 14, 21	Water Source	PCR
Flies, cockroaches, and rodents	32	0, 7, 14, 21	Floor	PCR
Footwear of the keepers	16	0, 7, 14, 21	Keepers´ Footwear	W/S/PCR
Pre-weaning swabs	36	-1	Floor	PCR
Post-weaning swabs	36	22	Floor	PCR

W = Willis; S = Sheater.

Statistical analysis

Statistical analysis of the data was performed using the FREQ and CORR procedure of the SAS 9.1.3 program.

Results and Discussion

Of the 141 stool samples analyzed in both coproparasitological test, in the two seasons evaluated, only four (2.84%) presented coccidium oocysts, which came from three of the 36 sows analyzed. (Table 2).

The results demonstrate that the sows rarely excrete or do not excrete *C. suis* oocysts, which is consistent with the findings by Linhares et al. (2012). In this study, *C. suis* positivity was observed in the two seasons evaluated, but with a very low occurrence. Sotiraki et al. (2008) affirms that the sows do not play an important role in the transmission of *C. suis* to the litters, a reiterated observation in the present study, where only one litter of three with positive sows showed simultaneous positivity in the piglets. However, even in low quantities, oocysts can become infectious and contaminate the entire environment and consequently litter, especially when there are hygienic-sanitary (León & Borges, 2009) and handling problems.

Of the 183 stool samples collected in the cage environment, coccidia oocysts were found in 37 (20.2%), of which 31 were from the litters and six were from the sows (Table 2). The 35 fecal samples positive for *C. suis* by coproparasitological methods were tested by PCR for confirmation, with 11 (31.43%) samples being positive. In two samples, it was not possible to perform the PCR because of insufficient fecal material.

Table 2
Results of coproparasitological and molecular exams of sow, litter, sow and litter cages, drinking water, winter and summer control and experimental groups for the detection of coccides in swine farm

Sample/Day/No. positives	W	S	P	W	S	P	W	S	P	W	S	P	W	S	P
	-5			0			7			14			21		
Sow CON/WI	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0
Sow TOL/WI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sow CON/SU	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Sow TOL/SU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Litter CON/WI	-	-	-	-	-	0	-	-	0	-	-	3	-	-	0
Litter TOL/WI	-	-	-	-	-	0	-	-	3	-	-	4	-	-	3
Litter CON/SUM	-	-	-	-	-	0	-	-	0	-	-	0	-	-	0
Litter TOL/SUM	-	-	-	-	-	0	-	-	0	-	-	0	-	-	0
Sow cage CON/WI	-	-	-	0	0	-	0	0	0	2	2	0	1	1	0
Sow cage TOL/WI	-	-	-	0	0	-	0	0	-	0	0	0	3	3	0
Sow cage CON/SU	-	-	-	0	0	-	0	0	-	0	0	-	0	0	-
Sow cage TOL/SU	-	-	-	0	0	-	0	0	-	0	0	-	0	0	-
Litter cage CON/WI	-	-	-	0	0	0	2	2	2	5	5	5	4	4	4
Litter cage TOL/WI	-	-	-	0	0	-	0	0	-	1	1	1	6	6	6
Litter cage CON/SU	-	-	-	0	0	0	0	0	0	3	3	3	4	4	4
Litter cage TOL/SU	-	-	-	0	0	0	2	2	2	0	0	0	3	3	3
Drinking water CON/WI	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0
Drinking water TOL/WI	-	-	1	-	-	0	-	-	1	-	-	0	-	-	1
Drinking water CON/SU	-	-	3	-	-	4	-	-	1	-	-	1	-	-	1
Drinking water TOL/SU	-	-	2	-	-	0	-	-	0	-	-	0	-	-	0

TOL: Toltrazuril; CON: Control Group; W: Willis; S: Sheather; P: PCR; WI: Winter; SU: summer.

Analyzing the positivity for *C. suis* between the control and treated group and between the season of the year for each period, for the samples collected from the environment (cages), no significant difference was observed ($p > 0.05$); that is, the prophylactic use of toltrazuril or the season of the year in which collections were performed did not interfere with the occurrence of the protozoan, reinforcing the need to perform coproparasitological examinations before using the drug.

Of the feces samples from the sows collected in the cage environment, only two samples had already been positive when collected directly from the female's rectal ampoule. Possibly, the other samples of environmental feces from the sows that were positive, became positive due to environmental contamination. This is because feces from sows and litters are all accumulated in the same place, and cross-contamination can easily occur, once they have been observed litters that showed positivity in the feces of

the environment and also in the rectal swabs, confirming that the infected animals were the litters and not the mothers.

Of the 144 samples of the pool of rectal swabs collected from each litter in the two seasons evaluated, 13 (9.03%) were positive for *C. suis* by the PCR technique and corresponded to nine of the 36 (25%) litters analyzed from litters aged between seven and 21 days.

Of the 184 water samples from the cage drinkers, 7.60% (14/184) were positive for C. suis as analyzed using PCR (Table 2). When we analyze the results obtained by the water analysis, we observe that the protozoan was present in the water of the animals' drinking fountains, both in the treated group and in the control group in summer and only in the treated group in winter, which may constitute a transmission route. The drinking fountain used in the facilities allowed the accumulation of water in a container, thus creating a means for the development of the parasite and facilitating its transmission, as was not constantly renewed. The correlation between the presence of *C. suis* in the drinking water and the presence of the agent in the litter was null in the summer and 36% in the winter, being considered a low association. In this case, the presence of the protozoan in the water did not necessarily mean that the animals were contaminated by this route.

There was no positivity in the samples of flies (35) and cockroaches (10) collected during the summer, in the molecular test used. No rodents were captured during the survey. The feces residues and organic matter samples collected in the sole of the attendants' shoes were all negative.

All samples collected in each cage, in the pre-accommodation period and after cleaning and disinfection, to detect possible residual oocysts in the facilities showed negative results.

All the samples of flies and cockroaches collected were negative, suggesting that they did not act as vectors. However, considering that the number of insects collected was insignificant (45), perhaps this result was underestimated. Similarly, the results show that the attendants' shoes did not constitute a means of transmission, since all samples were negative.

In the present work, the occurrence of C. suis was observed only in the winter period, and not in the summer period, in all the samples collected directly from the piglets rectal ampoule, with positivity for the referred protozoan, both in the treated group and in the control group, with greater positivity in the treated group.

Meyer et al. (1999) carried out a study in Germany and observed greater excretion in the summer, with 66.3% of the litters positive, when compared to other seasons. Despite the temperatures outside the facilities being lower in this station, the temperatures tend to get higher (32 to 35°C) inside the maternity wards, especially in the piglets creep area, due to the presence of the heating lamps, favoring more rapid sporulation of the oocysts and the continuation of the protozoan cycle (Paiva, 1996).

During winter, less attention may be paid by the keepers and producers concerning management, since, theoretically, it is a time when there is no evident presence of parasites and its effects on animals. In this

way, the lamps remain on most of the time, and the animals are more crowded, thus favoring contact between them, and the retention of heat inside the skimmer; this creates a warmer and more conducive environment for the sporulation of oocysts, compared to summer, when higher temperatures occur externally, although the is little crowding of the animals inside the facilities and the lamps stay on for a shorter time, not generating an internal heat that is favorable to the protozoan.

Using excretion of *C. suis* oocysts as a criterion, no significant difference ($p>0,05$) was observed between animals treated and those not treated with toltrazuril, corroborating Mundt et al. (2005) and Lippke et al. (2011) findings, wherein also a difference was not observed in the occurrence of oocysts between the control group and the group treated with toltrazuril. It must be taken into account that in the studied farm, cases of clinical coccidiosis were not so common and the animals were not excessively parasitized. For Maes et al. (2007), it is recommended to perform the coproparasitological examination before the start of routine treatment, because in farms that do not show clinical signs of coccidiosis and neither the expressive presence of *C. suis*, the benefits of this therapy do not outweigh the cost. It must be taken into account that in the studied farm, cases of clinical coccidiosis were not so common and the animals were not excessively parasitized.

In the present study, although the material in the facilities was solid (cement), all samples collected in each cage were negative. This result may be related to the fact that before the accommodation and after the end of this period and subsequent cleaning and disinfection, the remaining feces and organic matter residues were practically imperceptible, therefore the amount of material that was recovered with the swab became scarce. Mundt et al. (2005), when analyzing different types of floors used in the installations, observed that the type of floor used and the disinfection performed did not affect the occurrence of *C. suis* oocysts.

When analyzing the results obtained in the winter experiment, where a significant difference was observed and a greater positivity was found in the treated group, it was suggested to the owner to suspend the prophylactic toltrazuril, to avoid the pressure of resistant parasites' selection, and for economic reasons.

Thus, during the entire period from the end of the winter experiment (July) to the beginning of the summer experiment (January), the animals in the herd no longer received prophylactic toltrazuril treatment. Even with this suspension, when starting the summer experiment in February, no piglets were found with *C. suis* oocysts, both in the treated and control groups.

The prophylactic use of drugs with anticoccidial action in litters during the first week of life is already routine in most pig farms, even without the knowledge of the real need. The use of these drugs, in addition to being costly, can stimulate the selection of resistant parasites.

Conclusion

C. suis is not widely disseminated within the studied maternity environment, being observed more frequently during the winter.

Sows, the environment of the cages in the pre and post housing as well as during housing, flies, and cockroaches, and material contained in the keeper's footwear, were not the disseminators of C. suis for the litter. It is suggested that the water present in the drinking fountains of each cage could be the most likely route of transmission.

Treatment based on toltrazuril did not interfere with the occurrence of protozoa in animals. In this case, treating the animals or not, did not determine the occurrence or absence of the said protozoan.

Acknowledgment

CAPES - Higher Education Personnel Improvement Coordination

References

Adams, H. R. (2003). *Farmacologia e terapêutica em medicina veterinária.* Guanabara-Koogan.

Calderaro, F. F., Baccaro, M. R., Moreno, A. M., Ferreira, A. J. P., Jerez, A. J., & Pena, H. J. F. (2001) Frequência de agentes causadores de enterites em leitões lactentes provenientes de sistemas de produção de suínos do estado de São Paulo. *Arquivo do Instituto Biológico, 68*(1), 29-34.

D'Alencar, A. S., Faustina, M. A. G., Sousa, D. P., Lima, M. M., & Alves, L. C. (2006). Infecção por helmintos e coccídios em criação de suínos de sistema confinado localizado no município de Camaragibe-PE. *Ciência Veterinária nos Trópicos, 9*(2/3), 79-86.

León, J. C. P., & Borges, N. S. (2018). Aspectos de la dinámica de infección de Cystoisospora suis en lechones lactantes de una granja piloto del estado Carabobo, Venezuela. *Revista Científica, 28*(1), 42-51.

Linhares, G. F. C., Sobestiansky, J., Linhares, D., Barcellos, D., Moreno, A. M., & Matos, M. P. C. (2012). *Doenças dos Suínos.* Cânone Editorial.

Lippke, R. T., Borowski, S. M., Marques, S. M. T., Paesi, S. O., Almeida, L. L., Moreno, A. M., Corbellini, L. G., & Barcellos, D. E. S. N. (2011). Matched case-control study evaluating the frequency of the main agents associated with neonatal diarrhea in piglets. *Pesquisa Veterinária Brasileira, 31*(6), 505-510. doi: 10.1590/S0100-736X2011000600008

Maes, D., Vyt, P., Rabaes, P., & Gevaert, D. (2007). Effects of toltrazuril on the growth of piglets in herds without clinical isosporosis. *The Veterinary Journal, 173*(1), 197-199. doi: 10.1016/j.tvjl.2005.07.002

Meyer, C., Joachim, A., & Daugschies, A. (1999). Ocurrence of Isospora suis in larger piglet production units and on specialized piglet rearing farms. *Veterinary Parasitology, 82*(4), 277-284. doi: 10.1016/S0304-4017(99)00027-8

Mundt, H. C., Cohnen, A., Daugschies, A., Joachim, A., Prosl, H., Schmäschke, R., & Westphal, B. (2005). Ocurrence of Isospora suis in Germany, Switzerland and Austria. *Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health, 52*(2), 93-97. doi: 10.1111/j.1439-0450.2005.00824.x

Mundt, H. C., Joaquim, A., Becka, M., & Daugschies, A. (2006). *Isospora suis:* an experimental model for mammalian intestinal coccidiosis. *Parasitology Research, 98*(2), 167-175. doi: 10.1007/s00436-005-003 0-x

Nishi, S. M., Gennari, S. M., Lisboa, M. N. T. S., Silvestrim, A., Caproni, L., Jr., & Humehara, O. (2000). Parasitas intestinais em suínos confinados nos estados de São Paulo e Minas Gerais. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 67*(2), 199-203.

Paiva, D. P. de. (1996). *Suinocultura dinâmica - Isosporose suína.* (Periódico Técnico-Informativo). EMBRAPA-CNPSA.

Ruiz, V. L. A., Bersano, J. G., Carvalho, A. F., Catroxo, M. H. B., Chiebaod, P., Gregori, F., Miyashiro, S., Nassar, A. F. C., Oliveira, T. M. F. S., Ogata, R. A., Scarcelli, E. P., & Tonietti, P. O. (2016). Case-control study of pathogens involved in piglet diarrhea. *BMC Research Notes, 9*(22), 1-7. doi: 10.1186/s13104-01 5-1751-2

Samarasinghe, B., Johnson, J., & Ryan, U. (2008). Phylogenetic analysis of *Cystoisospora* species at the rRNA ITS1 locus and development of a PCR-RFLP assay. *Experimental Parasitology, 118*(4), 592-595. doi: 10.1016/j.exppara.2007.10.015

Sartor, A. A., Bellato, V., Souza, A. P. de, & Cantelli, C. R. (2007). Prevalência das espécies de Eimeria Schneider, 1875 e *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) parasitas de suínos do município de Videira, SC, Brasil. *Revista de Ciências Agroveterinárias, 6*(1), 38-43.

Sistema Meteorológico do Paraná (2019). *Dados meteorológicos de Palotina - PR.* SIMEPAR.

Sotiraki, S., Roepstorff, A., Nielsen, J. P., Maddox-Hyttel, C., Enoe, C., Boes, J., Murrell, K. D., & Thamsborg, S. M. (2008). Population dynamics and intra-litter transmission patterns of *Isospora suis* in suckling under on-farm conditions. *Parasitology, 135*(4), 395-405. doi: 10.1017/S0031182007003952

Spinosa, H. S., Górniak, S. L., & Bernardi, M. M. (2002). *Farmacologia aplicada à medicina veterinária.* Guanabara-Koogan.