RESEARCH

Identification of *Sarcocystis* spp. in wild boars (*Sus scrofa*) from Argentina

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Abstract

Sarcocystis spp. are intracellular protozoan parasites with an obligatory heteroxenous life cycle. The objective of this study is to identify *Sarcocystis* spp. in wild boar muscles from Argentina by light and transmission electron microscopy and molecular characterization. Muscle samples from diaphragm, tongue, masseter, intercostals, heart, and forelimbs of 240 wild boars were analyzed. Of the animals, 48.3% (116/240) were positive for sarcocysts by light microscopy, whereas 45.8% (110/240) were positive for *Sarcocystis* spp. by PCR targeting 18S rRNA fragment. These samples were subjected to a specific PCR for *S. suihominis coxI* gene, 3.6% (4/110) of which were weak positives. Unfortunately, sequence analysis was inconclusive. This could be related to a potentially low *S. suihominis* cyst load in the samples, or to an incomplete primer matching with the South American *S. suihominis* sequences. Seventeen individual sarcocysts were positive by PCR for the 18S rRNA fragment, whose sequences showed 99.75–100% identity with each other and with previously reported *S. miescheriana* sequences. A total of 21 cysts collected from 11 muscle samples and analyzed by TEM presented a cyst wall type compatible with *S. miescheriana*, and one cyst presented an ultrastructure compatible with *S. suihominis*. The latter came from a sample that also contained *S. miescheriana* cysts, indicating that the animal was co-infected. This is the first study that provides infection rates and describes and identifies morphological and molecular features of *Sarcocystis* spp. cysts in wild boars from South America.

Keywords *Sarcocystis suihominis* · *Sarcocystis miescheriana* · TEM · PCR sequencing · South America

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Introduction

Sarcocystis spp. are intracellular protozoan parasites with an obligatory heteroxenous life cycle, which mostly use herbivores as intermediate hosts (IH) and carnivores and/or omnivores as definitive hosts (DH). Oocysts and mature sporocysts formation occur in the DH's intestinal mucosa, whereas sarcocysts are formed by asexual reproduction in striated muscle of IH (Tenter 1995; Dubey et al. 2016). There are two *Sarcocystis* spp. properly described and known to infect swine as IH: *S. miescheriana* and *S. suihominis*, canids, and primates (human and non-human) being DH, respectively. A third species has been named *S. porcifelis* (with felids as putative DH), which has been considered to infect swine, although its existence and validity is still uncertain (Heydorn 1977; Avapal et al. 2004; Dubey et al. 2016). Swine become infected by ingesting food and/or water contaminated with sporulated oocysts or sporocysts, whereas the DH is infected by the ingestion of muscles harboring mature cysts of *Sarcocystis* spp.

So far, experimental infections have been carried out only in domestic pigs, evidencing that ingestion of low dose sporocysts, of either *S. suihominis* or *S. miescheriana*, generally results in subclinical infection (Heydorn 1977; Barrows et al. 1982; Caspari et al. 2011). Nevertheless, if a high dose of sporocysts is administered, it may cause symptoms like weight loss, dermatitis, dyspnea, skin purpura, muscle tremors, miscarriages, and even death (Calero-Bernal et al. 2016; Dubey et al. 2016). On the other hand, human infections are acquired by ingesting cysts present in raw or undercooked swine meat and are frequently characterized by diffuse gastrointestinal symptoms, such as diarrhea and stomachache (Fayer et al. 2015; Dubey et al. 2016; Rosenthal 2021).

The morphological characterization of *Sarcocystis* spp. could be partially achieved through direct light microscopy and histopathology, evidencing the sarcocyst's wall thickness and in some cases the type of cyst wall protrusions (Dubey et al. 2016). The use of scanning and transmission electron microscopy (SEM and TEM, respectively) is highly important since each *Sarcocystis* species produces a sarcocyst wall with unique ultrastructural features. The form and type of cyst wall protrusions allow differentiating species. There are more than 42 types and several subtypes of TEM sarcocyst walls described, related to species or groups of species (Dubey et al. 2016).

Molecular studies (PCR assays and sequencing of potential genetic markers) have recently allowed the identification of several *Sarcocystis* species, and they are currently considered as some of the most efficient, precise, and reliable methods (Gjerde 2013; Moré et al. 2013; Gazzonis et al. 2019; Huang et al. 2019; Chauhan et al. 2020; Prakas et al. 2020; Rubiola et al. 2020; Rosenthal 2021). Likewise, phylogenetic studies based on markers such as *18S rRNA* (18S ribosomal RNA), *28S rRNA* (28S ribosomal RNA), and *coxI* (mitochondrial cytochrome c oxidase subunit I) have revealed that both *S. miescheriana* and *S. suihominis* are sister species (Gazzonis et al. 2019; Huang et al. 2019; Prakas et al. 2020). Although *18S rRNA* sequencing is widely used both in the identification of diverse *Sarcocystis* species from different livestock and in reconstructing their phylogenetic relationships, its discriminatory power is not efficient due to its highly conserved nature, especially when it comes to closely related species (Caspari et al. 2011; Gjerde 2013; Moré et al. 2013; Prakas et al. 2020). For that reason, partial *coxI* sequences have resulted in a more suitable molecular marker to detect differences between species which are phylogenetically related (Gjerde 2013; Gazzonis et al. 2019; Prakas et al. 2020).

Swine sarcocystosis is widely distributed, with a prevalence from 30 to 50% in certain countries of Europe and Asia (Claveria et al. 2001; Imre et al. 2017; Huang et al. 2019; Januskevicius et al. 2019). A higher prevalence was recently reported in India, reaching almost 82% (Chauhan et al. 2020). There are few studies worldwide about the detection of *Sarcocystis* spp. in wild boars which, in most cases, revealed higher prevalence than in domestic pigs (Dubey et al. 2016). The latest studies of *Sarcocystis* spp. in wild boars showed infection rates ranging from 49% in the USA to 97% in Italy (Calero-Bernal et al. 2015, 2016; Coelho et al. 2015; Imre et al. 2017; Gazzonis et al. 2019; Prakas et al. 2020). Regarding the zoonotic species in domestic pigs, previous studies showed a prevalence ranging from 0.8% (5/600) in Japan (Saito et al. 1998), 8.8% (5/57) in India (Chauhan et al. 2020), and 17.1% (13/76) in China (Huang et al. 2019). Prevalence in wild boars was much lower: in Spain, it was identified in 12.5% (1/8) (Calero-Bernal et al. 2016) and in Italy in 1% (1/100) (Gazzonis et al. 2019) of the sequenced samples, and they were not found in studies carried out in Portugal (Coelho et al. 2015) or in Latvia (Prakas et al. 2020). There are no published studies concerning the morphological and molecular characterization of *Sarcocystis* spp. in swine from South America.

The aim of this study is to identify and characterize *Sarcocystis* spp. present in muscles from wild boars from Argentina, by light and transmission electron microscopy and by molecular characterization based on the *18S rRNA* and *coxI* genes. This is the first study that provides infection rates and describes and identifies morphological and molecular features of *Sarcocystis* spp. cysts in wild boars from South America.

Material and methods

Samples

Muscle samples from a total of 240 hunted free-range wild boars were collected by different research groups and sent refrigerated to the laboratory (LAINPA, FCV-UNLP). Both the sampling and their transport were conducted under national and provincial regulations and with their respective permissions. The animal location was classified according to three study regions, based on similar geoenvironmental conditions, such as landscape, vegetation, soil, climate, and fauna interactions and named *Pampeana-Litoral* (*PL*, *n* = 56), which included districts from Buenos Aires, La Pampa, Entre Ríos, and Corrientes provinces; *Northwest Patagonian* (*NWP*, *n* = 94), which included districts from Neuquén and west of Rio Negro provinces; and *Northeast Patagonian* (*NEP*, *n* = 90) which included areas from the east of Río Negro and south of Buenos Aires provinces (Fig. 1). Two hundred and forty animals were sampled, 113 of which were processed as pooled muscles of each animal (including some of the following: diaphragm, tongue, masseter, intercostals, heart, and forelimbs). The remaining 127 samples were processed as a single muscle homogenate, being 76
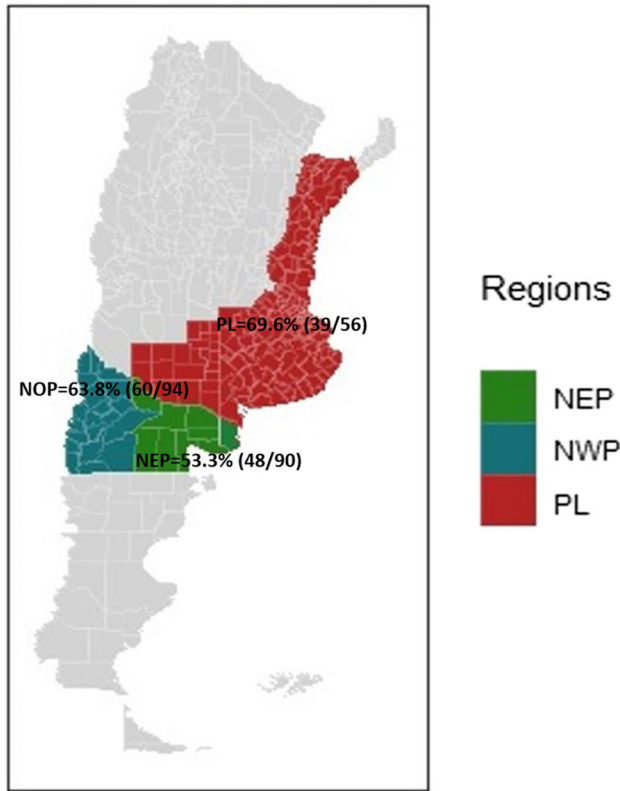
Sampling locations

Fig. 1 Sampling areas and *Sarcocystis* sp. positivity in wild boars in Argentina: Pampeana-Litoral (PL-n = 56): included localities from Buenos Aires, La Pampa, Entre Ríos, and Corrientes provinces; Northwest Patagonian (NWP-n = 94): included localities from Neuquén and west of Rio Negro provinces; and Northeast Patagonian (NEP-n = 90): included areas from the east of Río Negro and south of Buenos Aires provinces

from only diaphragm and 51 from other muscles (such as the tongue, masseter, intercostals, heart, and forelimbs).

Direct microscopic examination

The samples of each animal were processed following the methodology previously described by Moré et al. (2011). Briefly, 5 to 10 g of muscle was grounded in a tissue homogenizer with 50 ml phosphate buffered saline (PBS — pH 7.2), and then were filtered and centrifuged. Approximately 3-ml homogenate aliquots were placed in a Petri dish, diluted with PBS, and observed in an inverted microscope at 40 × magnification (Nikon, TMZ). An aliquot of each homogenate was collected in 1.5-ml DNAse-free tubes and preserved at − 20 °C for molecular studies. Samples containing at least one *Sarcocystis* sp. cyst (complete or portions of it) were considered positive. After collection, sarcocysts were photographed at 200 and 400 × magnification (Leica DM 2000 microscope).

Individualized sarcocysts were extracted from positive samples, stored in 1.5-ml DNAse-free tubes, and preserved at − 20 °C for subsequent molecular studies (PCR and sequencing). Ten sarcocysts were collected from each of 11 animal muscle homogenate samples and fixed in 2.5% glutaraldehyde for transmission electron microscopy (TEM).

TEM

Those samples were processed by standard techniques with a vacuum protocol and analyzed by TEM, as previously described (Moré et al. 2011). These procedures were carried out by the Electron Microscopy Central Service "Shin-Ichi Itagaki" from the FCV-UNLP using a JEM 1200 EX II (JEOL) transmission electron microscope.

DNA purification, PCR, and sequencing

DNA extraction was performed from each muscle homogenate sample (*n* = 240) obtained as described in point 2.2. In addition, some individual cysts were processed. The DNA was extracted from all samples using a commercial kit, following the manufacturer's instructions (Wizard genomics, Promega, USA). Each routine of DNA extraction was performed along with a process control sample (negative control, only using the kit solutions). A fragment of ~ 900 bp of the *Sarcocystis* spp. *18S rRNA* gene was amplified by PCR, using the primers SarcoFext and SarcoRext, as previously described (Moré et al. 2013). Additionally, homogenates and individual cysts positive by PCR were processed by a *S. suihominis*-specific PCR targeting a 1020-bp fragment from the *coxI* gene, using the SF8 and SR11 primers, as described before (Gazzonis et al. 2019). Each PCR routine included a negative control (DNA extraction process control sample), a no template control (NTC, ultrapure water), and a positive control (*S. miescheriana* DNA for *18S rRNA* or *S. suihominis* DNA— kindly provided by Luca Villa— for *coxI* PCR).

Amplicons from individual cyst of the *18S rRNA* PCR and from *coxI* PCR performed from muscle homogenates (with an estimated concentration of at least 40 ng/μl) were purified using a commercial kit according to the manufacturer's instructions (Wizard SV clean up system, Promega) and were submitted for Sanger sequencing to Macrogen Inc., South Korea (http://www.macrogen.com), along with both primers used for each amplification. The sequences obtained were aligned and analyzed using the Geneious software R9 version (https://www.geneious.com). The consensus sequences obtained were compared with others reported in GenBank by BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

The infection rates of *Sarcocystis* spp. (samples positive to optical microscopy and/or PCR) between the sampled regions were compared through a generalized linear model (logistic regression), after testing the effects of errors and the goodness of fit (Hosmer–Lemeshow test, *p*-value > 0.05). The degree of significance established was $p < 0.05$. Statistical analysis was performed using R software (RStudio Interface — version 2021.09.1 + 372).

Results

Direct microscopic examination and TEM

The 48.3% (116/240) of all homogenate samples analyzed were positive by direct microscopic examination. The majority of positive samples showed cyst portions of 150–300 μm, and the few complete cysts measured up to 600 μm (Fig. 2A). Bradyzoites were banana-shaped and measured approximately 12–15 μm (Fig. 2B).

Two cysts per sample could be observed and photographed by TEM (total $n = 22$), in all the cysts collected from the 11 diaphragm samples. Twenty one of these presented an ultrastructure compatible with *S. miescheriana* or TEM type 10 b (Dubey et al. 2016), and one showed a cyst wall ultrastructure compatible with *S. suihominis* or TEM type 31 (Dubey et al. 2016). *Sarcocystis miescheriana* cyst wall was between 3 and 5 μm thick and appeared radially striated; the protrusions measured up to 5 μm long and 1 μm wide and were palisade-like or tongue-shaped, close together, and with fine microtubules (Fig. 3A). The only *S. suihominis* cyst found showed a 3- to 6-μm-thick cyst wall with perpendicular villar protrusions measuring up to 13 μm long and 0.5 μm wide folded over the cyst surface and showed a thick ground substance layer (Fig. 3B).

PCR and sequencing

A total of 17 cysts, from 16 samples, were positive for the *18S rRNA* fragment PCR, and the amplicons obtained from them were suitable for subsequent sequencing. All of them came from different animals, except two, which corresponded to the same sample (J15-9). Partial *18S rRNA* gene sequences obtained in this study showed 99.75–100% identity with previously reported *S. miescheriana* sequences (MH404232, KT873760, KX929091, KT873751, and MT066112, among others). Sequencing results and BLAST comparisons are presented in Table 1. All 17 consensus sequences were uploaded in the GenBank, and they showed a 100% identity among them except one (TEC105), which was 99.8% (two single nucleotide polymorphisms).

The 240 homogenates were analyzed by *18S rRNA* fragment PCR and 110 were positive (45.8%). All of them were subjected to the specific PCR for *S. suihominis*, 3.6% (4/110) of which were weak positive and further processed by double PCR. The amplicons were submitted for sequencing, and the chromatograms showed poor quality, and no consensus sequence could be achieved. One of these samples (J15-9) showed *S. suihominis* cyst by TEM.

Statistical analysis

Out of all the processed samples, 147 (61.2%) were *Sarcocystis* spp., positive by direct microscopic examination and/or by PCR. In turn, the number of positive samples according to each region was 69.6% (39/56) for *PL*, 63.8% (60/94) for *NWP* and 53.3% (48/90) for *NEP* (Fig. 1). *Sarcocystis* spp. infection rates in wild boars from the *PL* and *NWP* regions were significantly higher than in *NEP*

Fig. 2 A *Sarcocystis* spp. cyst portion from wild boar muscle (200×). **B** *Sarcocystis* spp. bradyzoites released by compression of the cyst portion on **A** (400×)
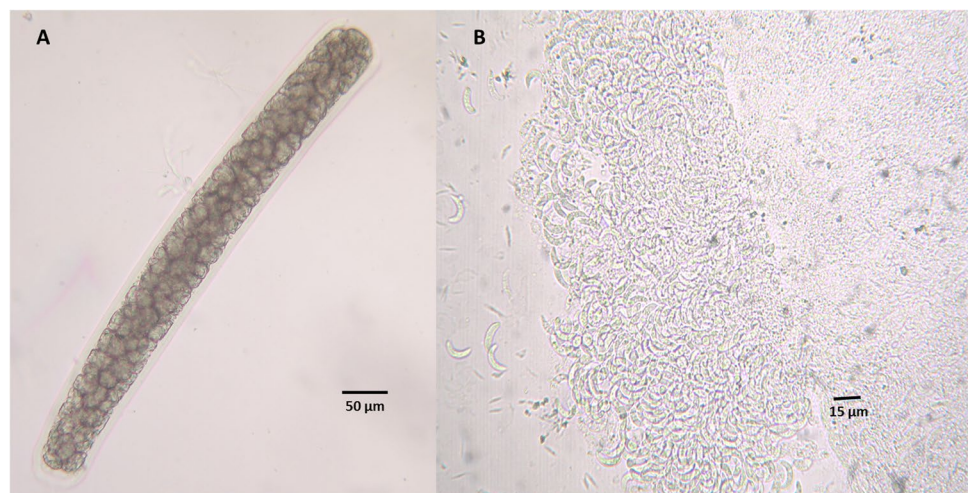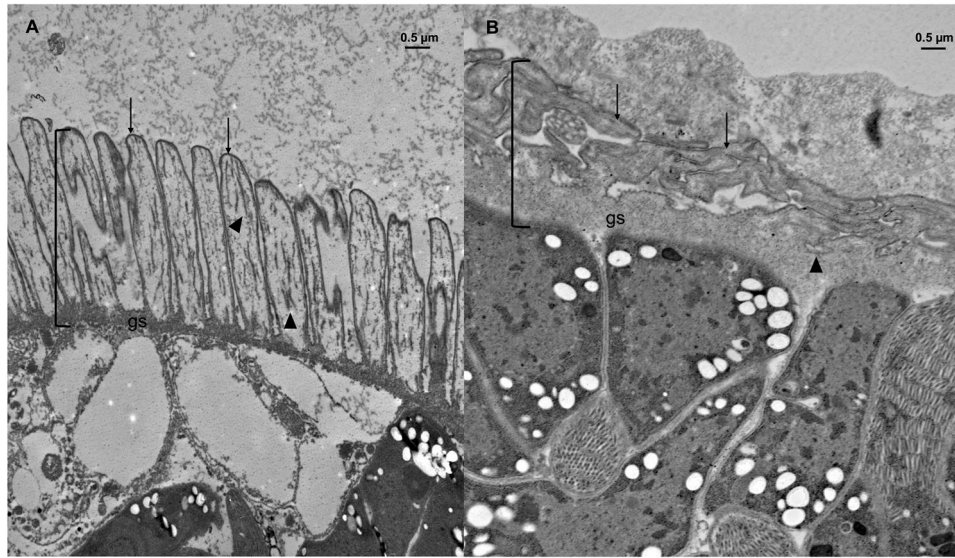
Fig. 3 A Longitudinal section of the wall of a *S. miescheriana* cyst from wild boar TEC 130 (TEM, 15,000×): bracket mark the cyst wall (3–5 µm thick), note the palisade-like protrusions (arrows, 3–5 µm long and 0.5–1 µm wide) containing microtubules (arrowhead) and a thin ground substance layer (gs, 0.2–0.4 µm). **B** Longitudinal section of the wall of a *S. suihominis* cyst from wild boar J15/9 from Argentina (TEM, 12,000×): bracket mark the cyst wall (3 to 5 µm thick), note the folded villar protrusions (arrows, 10–13 µm long and 0.3–0.5 µm wide) leaving the aspect of primary cyst wall invagination (arrowhead). The cyst wall showed a thick ground substance layer (gs, 0.5–1 µm)

Table 1 Sequencing results of 18S rRNA gene fragments from wild boar individual sarcocyst samples

Sample ID	Region	Muscle	Bp	BLASTn identity	GenBank accession number
J 15–5	NWP	Pool	834	100% *S. miescheriana* (#)	ON420292
J15-6	NWP	Pool	817	100% *S. miescheriana* (#)	ON420293
J15-8	NWP	Pool	826	100% *S. miescheriana* (#)	ON420294
J15-9 (cyst A)	NWP	Pool	832	100% *S. miescheriana* (#)	ON420295
J15-9 (cyst B)	NWP	Pool	661	100% *S. miescheriana* (#)	ON420296
TEC 72	NEP	Diaphragm	825	100% *S. miescheriana* (#)	ON420297
TEC 74	NEP	Diaphragm	820	100% *S. miescheriana* (#)	ON420298
TEC 87	NEP	Diaphragm	827	100% *S. miescheriana* (#)	ON420299
TEC 105	NEP	Diaphragm	786	99.75% *S. miescheriana* (#)	ON420300
TEC 130	PL	Diaphragm	799	100% *S. miescheriana* (#)	ON420301
TEC 152	NEP	Diaphragm	792	100% *S. miescheriana* (#)	ON420302
TEC 160	PL	Diaphragm	806	100% *S. miescheriana* (#)	ON420303
TEC 193	NEP	Other muscles	803	100% *S. miescheriana* (#)	ON420304
TEC 219	PL	Diaphragm	810	100% *S. miescheriana* (#)	ON420305
TEC 354	PL	Other muscles	878	100% *S. miescheriana* (#)	ON420306
TEC 355	PL	Other muscles	878	100% *S. miescheriana* (#)	ON420307
TEC 361	PL	Other muscles	881	100% *S. miescheriana* (#)	ON420308

PL Pampeana-Litoral, *NWP* Northwest Patagonian, *NEP* Northeast Patagonian, *Bp* sequence base pairs, # most representatives accession numbers of sequences retrieved by BLAST alignment (MH404232, KT873760, KX929091, KT873751, and MT066112)

(p-value < 0.05). A 48.3% (116/240) of the samples were positive by light microscopy and 45.8% (110/240) by the *18S rRNA* gene, with poor concordance between methods, since 37 of the 116 positive samples by light microscopy were negative for PCR, and 31 of the 110 positive samples for PCR were negative by light microscopy, and 79 were

positive by both methods. No significant differences in positivity were observed among the muscles analyzed, 61.9% from muscle pool (70/113), 63.2% (48/76) from diaphragm, and 56.9% (29/51) from other muscles.

Discussion

In this study, the rate of infection by *Sarcocystis* spp. was determined in free-range wild boars from three regions of Argentina using direct microscopic examination, molecular techniques, and TEM. The 61.2% (147/240) of the studied animals were found to present *Sarcocystis* spp. infection. Similar results had been reported from wild boars in some European countries, where a prevalence of 72.7%, 73.8%, and 60.4% has been described in Spain (Calero-Bernal et al. 2016), Portugal (Coelho et al. 2015), and Romania (Imre et al. 2017), respectively. A higher prevalence was reported in Italy (97%), which could be related to the use of a more sensitive detection method (Gazzonis et al. 2019). Additionally, in the mentioned studies, *S. miescheriana* was detected in most samples coinciding with the present study, where *S. miescheriana* was present in the 17 single-cyst samples amplified and sequenced. It is possible to assume, as reported by other studies, that *S. miescheriana* is present in most (or even all) of the *Sarcocystis* spp.-infected wild boars from Argentina. The proportion of wild boars infected with *Sarcocystis* spp. found in the *NWP* and *PL* region was significantly higher than those from *NEP*. This could be associated with the fact that the environmental conditions and natural distribution of canids (domestic and wild) in the *NWP* and *PL* regions are more suitable for the development of the biological cycle (more frequent interspecific contact) along with higher humidity, which could improve dispersion, viability, and infectivity of sporocysts. Regarding the different sampled muscles, no significant differences in the distribution of *Sarcocystis* spp. were found, which could suggest the lack of a specific tropism. Nevertheless, to confirm such hypothesis, different muscles from the same animals should be analyzed.

Mature *S. miescheriana* and *S. suihominis* sarcocysts differ in the cyst wall ultrastructure (Dubey et al. 2016). In the present study, 21 cysts collected and analyzed by TEM presented a cyst wall type compatible with *S. miescheriana*, and one showed an ultrastructure compatible with *S. suihominis* (Dubey et al. 2016). The latter came from a sample (J15/9) which also contained *S. miescheriana* cysts. Therefore, the animal was co-infected. These results show the relevance of performing cyst identification by TEM as complementary to molecular studies in order to achieve proper species identification based on ultrastructure characteristics of the cyst walls (Dubey et al. 2016). Moreover, since most animals were infected with *S. miescheriana*, it is highly probable

that animals infected with *S. suihominis* harbored both species in muscles, resulting in mixed sequencing analysis and difficulties to achieve proper molecular identification from muscle DNA, as was already suggested by others (Gazzonis et al. 2019).

The prevalence reported in this study based on *18S rRNA* PCR was slightly lower than that observed by light microscopy (45.8 versus 48.3%, respectively). Other studies using molecular detection methods have reported a higher prevalence (Calero-Bernal et al. 2016; Imre et al. 2017; Gazzonis et al. 2019). This could be related to the fact that in the present study, some samples were in an advanced decomposition state, which may have caused DNA degradation and consequent failure to achieve proper amplicons. The sequences of the 17 analyzed cysts showed a 99.8–100% identity with *S. miescheriana* sequences, with a high sequence homology among them and only one sample showing two single-base differences. The samples were from 16 animals distributed in the 3 sampling regions (6 from *NEP* and *PL* region and 5 from *NWP*), suggesting a low geographical species variation, as well as low intraspecific variation at the *18S* gene fragment sequenced. Similar results were found previously by Calero Bernal et al. (2016) and Gazzonis et al. (2019), when analyzing the *18S* gene sequences from *S. miescheriana* in wild boars in Europe.

Four of the 110 positives samples by *18S rRNA* PCR were weakly positive by a *S. suihominis*-specific PCR (Gazzonis et al. 2019), including the one where a *S. suihominis* cyst was observed by TEM (J15/9). To increase the amplicon concentration, a double PCR using primers SF8 and SR11 was performed, and the purified amplicons were submitted for sequencing. Unfortunately, the sequence quality was low, and no consensus sequence could be achieved. This could be related to a potentially low *S. suihominis* cyst load in the samples, as well as the mentioned coinfection with *S. miescheriana*, with an overrepresentation of the latter in terms of DNA concentration. Additionally, two single cysts amplified and sequenced, as well as cysts observed by TEM from the sample J15/9 (containing a *S. suihominis* cyst), were identified as *S. miescheriana*, suggesting a higher cyst burden of the latter species. Interestingly, the positive control provided by Luca Villa (named as WB9) yielded proper amplicons and good-quality sequences (Gazzonis et al. 2019). Therefore, it is possible that the *S. suihominis*-specific primers used, designed from an isolate from Europe, incompletely matched the sequences from the South American *S. suihominis* variant. More studies are needed to properly identify such potential sequence differences.

The *S. suihominis* prevalence detected in this study was slightly higher than in Spain and Italy where a single animal was identified as positive (Calero-Bernal et al. 2016; Gazzonis et al., 2019). Based on these studies and others performed during the last decade, the overall prevalence

of *S. suihominis* in swine is seemingly low in some European countries or even non-detected in others like Portugal, Romania, and USA (Calero-Bernal et al. 2015; Coelho et al. 2015; Imre et al. 2017). On the other hand, the highest prevalence has been reported in domestic pigs from India (Dubey et al. 2016; Chauhan et al. 2020). It has been suggested that the poor alimentary and sanitary conditions of populations may result in important environmental contamination with human feces, resulting in a high prevalence of *S. suihominis* in domestic pigs (Kaur et al. 2016). It is possible to suggest that wild boars, distributed in natural areas far from urban or suburban concentrations, would have less exposure to *S. suihominis* sporocysts present in human feces. This could explain the low prevalence detected in some European countries, whereas detection in this study could be related to the incursion of these animals in suburban or even urban environments of Argentina (Novillo and Ojeda 2008).

In conclusion, this study is the first *S. miescheriana* and *S. hominis* identification conducted in South America, indicating that the first species has the highest prevalence in wild boars from Argentina. Finally, future studies aimed to identify *S. suihominis* in the Americas should be conducted, focusing on obtaining species isolates and sequences that represent proper regional controls for the optimization of diagnostic tools. Application of reliable tests to monitor the presence of *S. suihominis* in domestic pig and wild boar meat destined for human consumption would be advisable, especially considering that raw or undercooked products are frequently consumed.

Author contribution Elisa Helman and Andrea Dellarupe processed the samples and performed microscopy and molecular studies as well as results analysis. They also wrote the manuscript.

Elizabeth Chang Reissig and Sabrina Cifuentes participated in sample collection, microscopy processing, and manuscript revision.

Gastón Moré was in charge of study design and molecular results analysis, and he wrote the manuscript.

All the authors reviewed the manuscript.

Availability of data and materials The sequences obtained in the frame of this study are reported and available at the GenBank (accession numbers ON420292- ON420208).

Declarations

Ethics approval Not applicable.

Consent to participate All the listed authors have made significant contributions to the study and agreed to participate.

Consent for publication All authors have read and approved this manuscript submission to be considered for publication.

Competing interests The authors declare no competing interests.

References

Avapal RS, Sharma JK, Juyal PD (2004) Pathological changes in *Sarcocystis* infection in domestic pigs (*Sus scrofa*). Vet J 168(3):358–361. https://doi.org/10.1016/j.tvjl.2003.11.006

Ballari SA, Barrios-García MN (2014) A review of wild boar *Sus scrofa* diet and factors affecting food selection in native and introduced ranges. Mamm Rev 44:124–134. https://doi.org/10.1111/mam.12015

Barrows PL, Prestwood AK, Green CE (1982) Experimental *Sarcocystis suicanis* infections: disease in growing pigs. Am J Vet Res 43(8):1409–1412

Calero-Bernal R, Verma SK, Oliveira S, Yang Y, Rosenthal BM, Dubey JP (2015) In the United States, negligible rates of zoonotic sarcocystosis occur in feral swine that, by contrast, frequently harbour infections with *Sarcocystis miescheriana*, a related parasite contracted from canids. Parasitology 142(4):549–556. https://doi.org/10.1017/S0031182014001553

Calero-Bernal R, Pérez-Martín JE, Reina D, Serrano FJ, Frontera E, Fuentes I, Dubey JP (2016) Detection of Zoonotic Protozoa *Toxoplasma gondii* and *Sarcocystis suihominis* in wild boars from Spain. Zoonoses Public Health 63(5):346–350. https://doi.org/10.1111/zph.12243

Caspari K, Grimm F, Kuhn N, Caspari NC, Basso W (2011) First report of naturally acquired clinical sarcocystosis in a pig breeding stock. Vet Parasitol 177(1–2):175–178. https://doi.org/10.1016/j.vetpar.2010.11.019

Chauhan RP, Kumari A, Nehra AK, Ram H, Garg R, Banerjee PS, Karikalan M, Sharma AK (2020) Genetic characterization and phylogenetic analysis of *Sarcocystis suihominis* infecting domestic pigs (*Sus scrofa*) in India. Parasitol Res 119(10):3347–3357. https://doi.org/10.1007/s00436-020-06857-3

Claveria FG, De La Pena C, Cruz-Flores MJ (2001) *Sarcocystis miescheriana* infection in domestic pigs (*Sus scrofa*) in the Philippines. J Parasitol 87(4):938–939. https://doi.org/10.1645/0022-3395(2001)087[0938:SMIIDP]2.0.CO;2

Coelho C, Gomes J, Inácio J, Amaro A, Mesquita JR, Pires I, Lopes AP, Vieira-Pinto M. (2015) Unraveling *Sarcocystis miescheriana* and *Sarcocystis suihominis* infections in wild boar. Vet Parasitol 15:212(3–4):100–4. https://doi.org/10.1016/j.vetpar.2015.08.015

Dubey JP, Calero-Bernal R, Rosenthal BM, Speer CA, Fayer R (2016). Sarcocystosis of animals and humans (2nd ed.). CRC Press. https://doi.org/10.1201/b19184

Fayer R, Esposito DH, Dubey JP (2015) Human infections with *Sarcocystis* species. Clin Microbiol Rev 28(2):295–311. https://doi.org/10.1128/CMR.00113-14

Gazzonis AL, Gjerde B, Villa L, Minazzi S, Zanzani SA, Riccaboni P, Sironi G, Manfredi MT (2019) Prevalence and molecular characterisation of *Sarcocystis miescheriana* and *Sarcocystis suihominis* in wild boars (*Sus scrofa*) in Italy. Parasitol Res 118(4):1271–1287. https://doi.org/10.1007/s00436-019-06249-2

Gjerde B (2013) Phylogenetic relationships among *Sarcocystis* species in cervids, cattle and sheep inferred from the mitochondrial cytochrome c oxidase subunit I gene. Int J Parasitol 43(7):579–591. https://doi.org/10.1016/j.ijpara.2013.02.004

Heydorn AO (1977) [Life-cycle of Sarcosporidia. IX. Developmental cyclus of *Sarcocystis suihominis* n. spec]. Berl Munch Tierarztl Wochenschr 90(11):218–24

Huang Z, Ye Y, Zhang H, Deng S, Tao J, Hu J, Yang Y (2019) Morphological and molecular characterizations of *Sarcocystis miescheriana* and *Sarcocystis suihominis* in domestic pigs (*Sus scrofa*) in China. Parasitol Res 118(12):3491–3496. https://doi.org/10.1007/s00436-019-06521-5

Imre K, Sala C, Morar A, Imre M, Ciontu C, Chisăliță I, Dudu A, Matei M, Dărăbuș G (2017) Occurrence and first molecular characterization of *Sarcocystis* spp. in wild boars (*Sus scrofa*) and domestic pigs (*Sus scrofa domesticus*) in Romania: public health significance of the isolates. Acta Trop 167:191–195. https://doi.org/10.1016/j.actatropica.2016.12.038

Januskevicius V, Januskeviciene G, Prakas P, Butkauskas D, Petkevicius S (2019) Prevalence and intensity of *Sarcocystis* spp. infection in animals slaughtered for food in Lithuania. Vet Med 64, 2019 (04): 149–157. https://doi.org/10.17221/151/2017-VETMED

Kaur M, Singh BB, Sharma R, Gill JP (2016) Pervasive environmental contamination with human feces results in high prevalence of zoonotic *Sarcocystis* infection in pigs in the Punjab, India. J Parasitol 102:229–232. https://doi.org/10.1645/15-828

Moré G, Abrahamovich P, Jurado S, Bacigalupe D, Marin JC, Rambeaud M, Venturini L, Venturini MC (2011) Prevalence of *Sarcocystis* spp. in Argentinean cattle. Vet Parasitol 19;177(1–2):162–5. https://doi.org/10.1016/j.vetpar.2010.11.036

Moré G, Schares S, Maksimov A, Conraths FJ, Venturini MC, Schares G (2013) Development of a multiplex real time PCR to differentiate *Sarcocystis* spp. affecting cattle. Vet Parasitol 197(1–2):85–94. https://doi.org/10.1016/j.vetpar.2013.04.024

Novillo A, Ojeda RA (2008) The exotic mammals of Argentina. Biol Invasions 10:1333–1344. https://doi.org/10.1007/s10530-007-9208-8

Prakas P, Kirillova V, Dzerkale A, Kirjušina M, Butkauskas D, Gavarāne I, Rudaitytė-Lukošienė E, Šulinskas G (2020) First molecular characterization of *Sarcocystis miescheriana* in wild boars (*Sus scrofa*) from Latvia. Parasitol Res 119(11):3777–3783. https://doi.org/10.1007/s00436-020-06882-2

Rosenthal BM (2021) Zoonotic *Sarcocystis*. Res Vet Sci 136:151–157. https://doi.org/10.1016/j.rvsc.2021.02.008

Rubiola S, Civera T, Ferroglio E, Zanet S, Zaccaria T, Brossa S, Cipriani R, Chiesa F (2020) Molecular differentiation of cattle *Sarcocystis* spp. by multiplex PCR targeting 18S and COI genes following identification of *Sarcocystis hominis* in human stool samples. Food Waterborne Parasit 21;18:e00074. https://doi.org/10.1016/j.fawpar.2020.e00074

Saito M, Shibata Y, Ohno A, Kubo M, Shimura K, Itagaki H (1998) *Sarcocystis suihominis* detected for the first time from pigs in Japan. J Vet Med Sci 60(3):307–309. https://doi.org/10.1292/jvms.60.307

Tenter AM (1995) Current research on *Sarcocystis* species of domestic animals. Int J Parasitol 25(11):1311–1330. https://doi.org/10.1016/0020-7519(95)00068-d