Macroscopic sarcocystosis in a pig carcass from an Italian abattoir

Macroscopic sarcocystosis in a pig carcass from an Italian abattoir

Selene Rubiola (selene.rubiola@unito.it)
University of Turin
Linda Pasquariello
University of Turin
Felice Panebianco
University of Turin
Maria Teresa Capucchio
University of Turin
Elena Colombino
University of Turin
Federica Bordese
ASL Cuneo 1, S.C. Igiene degli Alimenti di Origine Animale
Enrique Giobbio
ASL Torino 5, S.C. Sanità Animale, Torino, Italy
Lisa Fioriello
ASL Torino 5, S.C. Sanità Animale, Torino, Italy
Silvia Braghin
ASL Torino 5, S.C. Sanità Animale, Torino, Italy
Weronika Korpysa-Dzirba
National Veterinary Research Institute in Pulawy
Mirosław Różycki
Poznań University of Life Science
Francesco Chiesa
University of Turin

Short Report

Keywords: Sarcocystis miescheriana, domestic pig, macroscopic sarcocystosis, cox1 mtDNA gene, 18S rRNA, carcass condemnation

Posted Date: April 13th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2793791/v1

Version of Record: A version of this preprint was published at Veterinary Research Communications on May 11th, 2023. See the published version at https://doi.org/10.1007/s11259-023-10137-7.

Abstract

Different food-safety institutions, including the European Food Safety Authority, encourage monitoring and characterising *Sarcocystis* spp. in animals and foodstuffs; among meat-producing animals, domestic pigs (*Sus scrofa*) can host two different *Sarcocystis* spp., that is *Sarcocystis miescheriana* and *Sarcocystis suihominis*. Herein, we report for the first time the presence of macrocysts of *Sarcocystis miescheriana* in a domestic pig resulting in carcass condemnation. In June 2022, the carcass of a clinically healthy sow was condemned due to the detection of multifocal macroscopic white oval lesions. Affected muscle samples were submitted to histological and molecular analyses targeting the mtDNA *cox1* and 18S rRNA genes. At gross examination and histology, well demarcated, oval or elongated macrocysts up to 8 mm in length characterized by a calcified central core surrounded by fibrosis were detected. The molecular amplification and sequencing of the *cox1* mtDNA and 18S rRNA genes revealed the presence of *Sarcocystis miescheriana* DNA in all sampled macrocysts. Our study provides the first molecularly confirmed case of *Sarcocystis miescheriana* infection in a domestic pig in Italy. The present report highlights the need to increase data related to the occurrence and the prevalence of *Sarcocystis* spp. in meat-producing animals, and in wild and domestic pigs in particular, taking into account the zoonotic potential of *Sarcocystis suihominis* and the possible financial losses related to carcass discard due to macroscopic *Sarcocystis* spp. lesions.

1. Introduction

Sarcocystis is a neglected and emerging genus of intracellular protozoan parasites including more than 200 species infecting a wide range of vertebrates (Dubey et al., 2015). The two-host pray-predator life cycle of *Sarcocystis* spp. includes carnivores and omnivores as definitive hosts, which become infected through the ingestion of muscle tissue containing mature sarcocysts, and herbivores, omnivores or carnivores as intermediate hosts, which become infected ingesting food, feed and water contaminated with faeces containing oocysts or sporocysts (Gazzonis et al., 2019). Three zoonotic species, that is *S. suihominis*, *S. hominis* and *S. heydorni*, can infect humans due to the consumption of undercooked or raw beef or pork (Dubey et al., 2015).

Among meat-producing animals, domestic pigs (*Sus scrofa*) can host two *Sarcocystis* spp., that is *S. miescheriana* and *S. suihominis*, using canids and human and nonhuman primates, respectively, as definitive hosts. Humans, which become infected by eating raw or undercooked pork meat containing muscular *S. suihominis* cysts, can develop gastrointestinal symptoms including nausea, vomiting, stomachache and diarrhoea; nevertheless, most infections are paucisymptomatic or asymptomatic (Dubey et al., 2015). Both *S. miescheriana* and *S. suihominis*, usually cause subclinical infections in pigs. However, as proven by experimental infections, the ingestion of more than 1 million sporocysts can cause weight loss, thrombocytopenia, purpura of the skin, muscle tremors, dyspnoea, abortion and even the pig death (Daugschies et al., 1987). Actually, only one report of clinical sarcocystosis after natural infection has been described in pigs worldwide, determining the death of the affected domestic swine due to severe myocarditis; no gross lesions were observed at the necroscopy (Caspari et al., 2011).

Despite the lack of clinical cases in domestic swine, European surveys have shown a prevalence of *Sarcocystis* spp. in domestic pigs ranging from 3–43% (Dubey et al., 2015; Pereira & Bermejo, 1988); nevertheless, studies referring to the prevalence of sarcocystosis in domestic pigs reared in intensive indoor production system are rather dated, and most often did not distinguish between *S. miescheriana* and *S. suihominis*. Otherwise, the emphasis in recent years has been on prevalence studies in wild boars, showing higher infection rates than in domestic swine (Calero-Bernal et al., 2016; Coelho et al., 2015; Helman et al., 2022; Prakas et al., 2020). So far, no molecular investigations have been performed in Italy into the occurrence of *S. suihominis* and *S. miescheriana* in domestic swine.

Different food-safety institutions, including the European Food Safety Authority (EFSA), encourage monitoring and characterising *Sarcocystis* spp. in animals and foodstuffs (Taylor et al., 2010). However, while reports on bovine *Sarcocystis* spp. are growing, especially due to the fact that different *Sarcocystis* spp. have been associated with bovine eosinophilic myositis (Rubiola et al., 2020, 2021), there is no documentation of any species of *Sarcocystis* in pigs related to the presence of macroscopic sarcocysts causing carcass condemnation (Dubey et al., 2015). Furthermore, the quality of the meat of experimentally infected pigs, which has been investigated to estimate the economic impact of subclinical sarcocystosis, has not been reported to be reduced (Daugschies et al., 1987). Herein, the presence of macrocysts of *S. miescheriana* in a pig resulting in carcass condemnation is reported for the first time; furthermore, our study provides the first molecularly confirmed case of *S. miescheriana* infection in a domestic pig in Italy.

2. Materials And Methods

In June 2022, the carcass of a clinically healthy sow was condemned at slaughter due to the presence of multifocal macroscopic white lesions detected during the post-mortem meat inspection. The Large-White x Landrace sow was born in a commercial pig farm in Piedmont, North-West Italy. After weaning, the pig was purchased by an indoor farm located in the same area, where it was reared for four years until it was bought by a nearby fattening pig farm and sent to slaughter after three months. No signs of disease nor abnormalities in body weight were recorded during the lifetime. The sow carcass was condemned due to the extensive involvement of different muscles, including masseter, tongue, neck, oesophagus, diaphragm, shoulders and back muscles and skeletal muscles of the fore and hind limbs. Affected muscle samples were submitted by the slaughterhouse to the Department of Veterinary Sciences (University of Turin, IT) for etiological diagnosis.

A gross examination of the affected muscle samples was performed; tissues affected by lesions were fixed in 10% buffered formalin and stored at room temperature for histopathological examination. Fixed samples were trimmed, embedded in paraffin wax, sectioned at 4 µm, stained with haematoxylin and eosin (H&E) and examined using a light microscope.

Ten of the detected lesions were isolated, excised and stored at − 20°C for molecular examination. DNA extraction was performed using DNeasy Blood and Tissue Kit (Qiagen, Germany), according to the

manufacturer's tissue protocol. Additionally, ten grams of healthy muscle were collected from different portion of the muscle samples and processed as described by Morè et al. (2011) in order to obtain a muscle homogenate; the resulting pellet was resuspended in 20 ml of phosphate buffered saline (PBS) and observed in an inverted microscope at 40 × magnification (Nikon TMS).

All DNA samples were initially tested for the presence of *Taenia* spp. DNA by PCR targeting a fragment of the cytochrome C oxidase subunit I mitochondrial gene (mtDNA *cox1*) using primer pair JB3 - JB4.5 as described by Bowles et al. (1992), and for the presence of *Sarcocystis* spp. DNA by PCR using the *Sarcocystis* genus-specific primer pair SARf – SARr amplifying a small portion of the 18S rRNA gene, as described by Vangeel et al. (2007). Positive samples were subsequently further molecularly characterised targeting the partial mtDNA *cox1* gene with primers SF1 and SR11 (~ 1100 bp) as described by Gazzonis et al. (2019). The complete 18S rRNA gene of one out of ten samples was amplified using the primer sets ERIB1 - S2 (Barta et al., 1997; Fischer & Odening, 1998) and S3 - Primer BSarc (Fischer & Odening, 1998; Gjerde, 2014). The sequences of all primers used are reported in Table 1.

Table 1
Primers used in the present study to screen for the presence of *Taenia* spp. DNA, *Sarcocystis* spp. DNA (SARf - SARr) and to characterise the mtDNA *cox1* gene (SF1 - SR11) and the complete 18S rRNA gene (ERIB1 - S2, S3 - Primer BSarc).

Target	Gene	Primer name	Sequence 5' – 3'	References
Taenia spp.	*cox1*	JB3	TTT TTT GGG CAT CCT GAG GTT TAT	Bowles et al., 1992
		JB4.5	TAA AGA AAG AAC ATA ATG AAA ATG	Bowles et al., 1992
Sarcocystis spp.	18S	SARf	TGG CTA ATA CAT GCG CAA ATA	Vangeel et al., 2007
		SARr	AAC TTG AAT GAT CTA TCG CCA	Vangeel et al., 2007
		ERIB1	ACC TGG TTG ATC CTG CCA G	Barta et al., 1997
		S2	CTG ATC GTC TTC GAG CCC CTA	Fischer & Odening, 1998
		S3	TTG TTA AAG ACG AAC TAC TGC G	Fischer & Odening, 1998
		Primer BSarc	GAT CCT TCC GCA GGT TCA CCT AC	Gjerde, 2014
S. miescheriana	*cox1*	SF1	ATG GCG TAC AAC AAT CAT AAA GAA	Gjerde, 2013
		SR11	GGA AGT GGG CAA CAA TGT AAT A	Gazzonis et al., 2019

PCR products amplified using primers SARf - SARr, SF1 - SR11, ERIB1 - S2 and S3 - Primer BSarc were purified (ExoSAP-IT, Thermo Fisher Scientific, USA) and sequenced using the BigDye Terminator v1.1

Cycle Sequencing Kit (Thermo Fisher Scientific, USA); sequence analysis was performed on an Applied Biosystems SeqStudio Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA). Forward and reverse sequences, as well as the 18S rRNA overlapping sequences, were manually assembled into consensus sequences using the Alignment Explorer within MEGA X (Kumar et al., 2016). The nucleotide sequences were analysed using the BLASTN sequence similarity search at the NCBI database. Phylogenetic analyses of the mtDNA *Cox1* gene and the 18S rRNA gene sequences were conducted separately. With reference to the mtDNA *cox1* gene, a total of 70 partial sequences from 54 taxa were used in the analysis, including 9 out of 10 new sequences generated in the present study; as for the 18S rRNA gene, a total of 60 sequences from 54 taxa were used in the analysis, including the new sequence generated in the present study. Multiple alignments of the generated sequences and *Sarcocystis* spp. sequences retrieved from GenBank were obtained by using the ClustalW algorithm in MEGA X. The phylogenetic trees were reconstructed using the neighbor-joining method within MEGA X. As for the mtDNA *cox1*, sequences longer than 1020 bp were truncated so that the final alignment comprised 1020 positions with no gaps. In both analyses of the mtDNA *cox1* gene and the 18S rRNA gene, the coccidian parasite *Neospora caninum* was used as outgroup species to root the trees. The phylogeny was tested with the bootstrap method (1000 replicates). *Sarcocystis* spp. reference sequences included in the analyses are reported in the phylogenetic trees. The DNA Sequence Polymorphism (DnaSP) software (version 6.12.03) was used to calculate intraspecific genetic variation measurements.

Sequences generated in the present study were submitted in GenBank under accession numbers OQ472889 and OQ472068-OQ472077.

3. Results

At gross examination, skeletal muscles showed multifocal, white, well demarcated, oval or elongated lesions up to 8 mm in length and 3 mm in diameter described as macrocysts; most of the lesions appeared solid and released white necrotic material when squeezed. Macroscopic appearance of the affected muscle samples is shown in Fig. 1. Histologically, the three measured club-shaped cysts were 950–2900 x 600–900 µm in size. Most of them were characterized by a calcified central core surrounded by fibrosis and a slight non suppurative inflammatory reaction composed by lymphocytes, macrophages and rare plasma cells (Fig. 2). A thin rim of atrophic, degenerated muscle fibres was present at the periphery. Multifocal areas of fibrosis were also detected in the affected muscles.

All collected samples tested negative for the presence of *Taenia* spp. DNA. The healthy muscle homogenate tested negative for the presence of sarcocysts by direct microscopic examination. On the other hand, the initial screening of excised lesions for Sarcocystidae DNA by PCR assay targeting *Sarcocystis* genus revealed the presence of *Sarcocystis* spp. DNA in all sampled lesions. Sanger sequencing of the amplified products resulted in ~ 180 bp fragments showing 100% identity with each other and with *S. miescheriana* GenBank entries (accession no. MH404230, KX008292, JN256123, MT066104-MT066113). All the ten DNA samples corresponding to the lesions were therefore submitted

to the partial amplification and sequencing of the mtDNA *cox1* gene using primers SF1 - SR11 and one DNA sample was characterised at complete 18S rRNA gene.

The obtained 1869 bp-long 18S rDNA sequence showed 99.5–100% identity with *S. miescheriana* (accession no. MK867454, MH404232, MH404231, JN256123, MT066104-MT066113, MH404230) and 96.1–98.0% identity with *S. suihominis* (accession no. KP732433-KP732435, AF176936-AF176937, MH404229 and MK867455) (Fig. 3b). On the basis of mtDNA *cox1* gene, the newly obtained 1020–1073 bp-long sequences demonstrated 94.4–99.7% identity with *S. miesheriana* GenBank entries (accession no. MK867462-MK867464, LC349977-LC349980, MH404185-MH404227, MT070614-MT070635, OP681505-OP681509, ON211345- ON211346) and less than 80.4% identity with other *Sarcocystis* spp. sequences available in GenBank. Based on these results, it was confirmed that all excised macrocysts belonged to *S. miescheriana* (Fig. 3). One out of 10 *cox1* sequences was removed from further analysis due to the shorter length (716 bp). The phylogenetic analysis on the basis of *cox1* sequences showed close relationships of *S. miescheriana* sequences from the present study with *S. miescheriana* from Italian wild boar and from Latvian and Lithuanian wild boar (Fig. 3a). The 9 sequences represented 5 haplotypes (haplotype diversity: 0.861 ± 0,00760); 13 polymorphic (segregating) sites were detected among the 1020 nucleotide positions covered by all haplotypes. The sequence divergence among the generated *S. miescheriana* sequences ranged from 0.001 to 0.01%. While the average number of nucleotide differences within the newly obtained *S. miescheriana* sequences was 5.2, the average number of nucleotide differences between the generated *S. miescheriana* sequences (n = 9) and the homologous fragments of *S. miescheriana* isolated from Italian wild boars (n = 43; MH404185-MH404227), Lithuanian and Latvian wild boars (n = 22; MT070614-MT070635), Chinese pigs (n = 3; MK867462-MK867464) and Japanese wild boars (n = 4; LC349977-LC349980) corresponded to 13.2, 9.5, 33.4 and 51.4 respectively.

4. Discussion

Reports concerning the occurrence of *Sarcocystis* spp. in livestock in EU countries are scarce and difficult to interpret (Taylor et al., 2010). Regulation (EU) 2019/627, repealing regulation (EU) 854/2004, covers *Sarcocystis* spp. under the blanket term “zoonotic agents”, recommending their detection in beef and pork and stating that carcasses infected with parasites have to be declared unfit for human consumption. In this context, *Sarcocystis* spp. infections in domestic swine were not paid much attention in the veterinary field, as natural infections do not usually cause clinical symptoms nor gross lesions visible at slaughter (Dubey et al., 2015).

The current study reports for the first time the presence of disseminated macroscopic *S. miescheriana* cysts in a domestic pig, resulting in carcass condemnation. As stated by Dubey et al. (2015), there is no documentation about the economic impact of swine sarcocystosis; indeed, although swine carcass discard due to suspected sarcocystosis has been reported in the past, there was no associated evidence supporting the presence of any *Sarcocystis* spp. or macroscopic sarcocyst. It is therefore unclear whether sarcocysts were actually present or the lesions were misidentified (Dubey et al., 2015). Herein the presence of white, sometimes calcified cysts up to 8 mm in length is described. As shown in the present

report, the presence of macroscopic cysts can possibly involve the entire carcass, thereby leading to carcass condemnation and economic losses, in the absence of clinical signs. As previously hypothesized by Caspari et al. (2011), due to the high hygiene standards and close housing systems adopted by pig farms in the last decades, pigs natural immunisation with *S. miescheriana* might be decreased, thereby allowing the emergence of clinical cases or abnormal response of the host tissue to the infection.

The molecular identification and characterization of the etiological agent involved in the present report has allowed to rule out the presence of other parasites which might be associated to the detection of degenerated cysts or other macroscopic lesions, e.g. calcified cysticerci (González et al., 2006). The phylogenetic analysis on the basis of mtDNA *cox1* and 18S rRNA sequences and the intraspecific genetic variation analysis showed close relationships of *S. miescheriana* isolated in the present study and *S. miescheriana* isolated from Latvian, Lithuanian and Italian wild boars. On the other hand, our isolates were genetically distinct from *S. miescheriana* isolates originating from Chinese domestic pig and Japanese wild boars (Gazzonis et al., 2019; Huang et al., 2019), thereby showing the genetic similarity of the samples to be dependent upon their geographical distances as previously observed by Prakas et al. (2020).

Moreover, herein the first molecularly confirmed case of *S. miescheriana* infection in a domestic pig in Italy is reported. So far, no investigations into the occurrence and prevalence of *S. suihominis* and *S. miescheriana* have been conducted in domestic swine in Italy. Likewise, at EU level, emphasis in recent years has been placed upon prevalence studies in wild boars, feral pigs and free-ranging domestic pigs, while there are no recent investigations on the occurrence of sarcocystosis in domestic pigs kept under indoor intensive systems (Calero-Bernal et al., 2015, 2016; Helman et al., 2022; Kaur et al., 2016; Prakas et al., 2020). Our report stresses the need to increase data related to the occurrence of *Sarcocystis* spp. in wild boars and domestic pigs at national and international level, pointing out the possible presence of *Sarcocystis* spp. cycling in pigs raised in intensive conditions.

Although *S. miescheriana* cannot infect humans, and therefore is not a direct risk for the consumer of raw or undercooked meat, its detection highlights, on one hand, the possible economic damage related to carcass discard due to macroscopic *Sarcocystis* spp. sarcocysts, and on the other hand, the presence of a biosecurity breach. The infection source of the present case was not investigated, and therefore cannot be unequivocally stated; nevertheless, two owned dogs were recorded in the indoor farm where the sow was reared for four years. Thus, the domestic dogs are most likely the source of *S. miescheriana* sporocysts, which might have been introduced in pigpens despite the absence of shared areas.

In conclusion, to the best of our knowledge, our study reports the first molecularly confirmed case of *S. miescheriana* infection in a domestic pig in Italy; furthermore, it reports the first condemnation of a pig carcass due to the presence of disseminated *Sarcocystis* macrocysts. The present study highlights the need to increase data related to the occurrence and prevalence of *Sarcocystis* spp. in wild and domestic pigs, taking into account the zoonotic potential of *S. suihominis* and the possible financial losses related to carcass discard due to macroscopic *Sarcocystis* spp. cysts.

Declarations

Funding

This study received no external funding.

Competing interests

The authors declare that they have no conflict of interest.

Author Contributions

Selene Rubiola: Writing - original draft, Conceptualization, Investigation, Formal analysis. Linda Pasquariello: Writing - review & editing, Investigation. Felice Panebianco: Writing - review & editing, Investigation. Maria Teresa Capucchio: Writing - review & editing, Investigation. Elena Colombino: Writing - review & editing, Investigation. Federica Bordese: Writing - review & editing, Investigation. Enrique Giobbio: Writing - review & editing, Investigation. Lisa Fioriello: Writing - review & editing, Investigation. Silvia Braghin: Writing - review & editing, Investigation. Weronika Korpysa-Dzirba: Writing - review & editing, Investigation. Mirosław Różycki: Writing - review & editing, Investigation. Francesco Chiesa: Writing – review & editing, Conceptualization, Resources, Supervision, Funding acquisition, Project administration. All authors read and approved the final manuscript.

Data availability

The datasets generated in the present study are available in GenBank database with accession numbers OQ472889 and OQ472068-OQ472077.

Ethics approval

Not applicable.

References

1. Barta JR, Martin DS, Liberator PA, et al (1997) Phylogenetic Relationships among Eight Eimeria Species Infecting Domestic Fowl Inferred Using Complete Small Subunit Ribosomal DNA Sequences. The Journal of Parasitology 83:262–271. https://doi.org/10.2307/3284453
2. Bowles J, Blair D, McManus DP (1992) Genetic variants within the genus Echinococcus identified by mitochondrial DNA sequencing. Molecular and Biochemical Parasitology 54:165–173. https://doi.org/10.1016/0166-6851(92)90109-W
3. Calero-Bernal R, Pérez-Martín JE, Reina D, et al (2016) Detection of Zoonotic Protozoa Toxoplasma gondii and Sarcocystis suihominis in Wild Boars from Spain. Zoonoses and Public Health 63:346–350. https://doi.org/10.1111/zph.12243

4. Calero-Bernal R, Verma SK, Oliveira S, et al (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 142:549–556. https://doi.org/10.1017/S0031182014001553
5. Caspari K, Grimm F, Kühn N, et al (2011) First report of naturally acquired clinical sarcocystosis in a pig breeding stock. Veterinary Parasitology 177:175–178. https://doi.org/10.1016/j.vetpar.2010.11.019
6. Coelho C, Gomes J, Inácio J, et al (2015) Unraveling Sarcocystis miescheriana and Sarcocystis suihominis infections in wild boar. Veterinary Parasitology 212:100–104. https://doi.org/10.1016/j.vetpar.2015.08.015
7. Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying Down Uniform Practical Arrangements for the Performance of Official Controls on Products of Animal Origin Intended for Human Consumption in Accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and Amending Commission Regulation (EC) No2074/2005 as Regards Official Controls. 50. Accessed on February 22, 2023 Available online: https://eur-lex.europa.eu/eli/reg_impl/2019/627/oj
8. Daugschies A, Rommel M, Schnieder T, et al (1987) Effects of Sarcocystis miescheriana infection on carcass weight and meat quality of halothane-tested fattening pigs. Veterinary Parasitology 25:19–31. https://doi.org/10.1016/0304-4017(87)90061-6
9. Dubey JP, Calero-Bernal R, Rosenthal BM, et al (2015) Sarcocystosis of Animals and Humans. CRC Press
10. Fischer S, Odening K (1998) Characterization of Bovine Sarcocystis Species by Analysis of Their 18S Ribosomal DNA Sequences. The Journal of Parasitology 84:50–54. https://doi.org/10.2307/3284529
11. Gazzonis AL, Gjerde B, Villa L, et al (2019) Prevalence and molecular characterisation of Sarcocystis miescheriana and Sarcocystis suihominis in wild boars (Sus scrofa) in Italy. Parasitol Res 118:1271–1287. https://doi.org/10.1007/s00436-019-06249-2
12. Gjerde B (2014) Morphological and molecular characteristics of four Sarcocystis spp. in Canadian moose (Alces alces), including Sarcocystis taeniata n. sp. Parasitol Res 113:1591–1604. https://doi.org/10.1007/s00436-014-3806-z
13. Gjerde B (2013) Phylogenetic relationships among Sarcocystis species in cervids, cattle and sheep inferred from the mitochondrial cytochrome c oxidase subunit I gene. International Journal for Parasitology 43:579–591. https://doi.org/10.1016/j.ijpara.2013.02.004
14. González LM, Villalobos N, Montero E, et al (2006) Differential molecular identification of Taeniid spp. and Sarcocystis spp. cysts isolated from infected pigs and cattle. Veterinary Parasitology 142:95–101. https://doi.org/10.1016/j.vetpar.2006.06.009
15. Helman E, Dellarupe A, Cifuentes S, et al (2022) Identification of Sarcocystis spp. in wild boars (Sus scrofa) from Argentina. Parasitol Res. https://doi.org/10.1007/s00436-022-07743-w

16. Huang Z, Ye Y, Zhang H, et al (2019) Morphological and molecular characterizations of Sarcocystis miescheriana and Sarcocystis suihominis in domestic pigs (Sus scrofa) in China. Parasitol Res 118:3491–3496. https://doi.org/10.1007/s00436-019-06521-5
17. Kaur M, Singh BB, Sharma R, Gill JPS (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
18. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33:1870–1874. https://doi.org/10.1093/molbev/msw054
19. Moré G, Maksimov A, Conraths FJ, Schares G (2016) Molecular identification of Sarcocystis spp. in foxes (Vulpes vulpes) and raccoon dogs (Nyctereutes procyonoides) from Germany. Veterinary Parasitology 220:9–14. https://doi.org/10.1016/j.vetpar.2016.02.011
20. Pereira A, Bermejo M (1988) Prevalence of Sarcocystis cyst in pigs and sheep in Spain. Veterinary Parasitology 27:353–355. https://doi.org/10.1016/0304-4017(88)90049-0
21. Prakas P, Kirillova V, Dzerkale A, et al (2020) First molecular characterization of Sarcocystis miescheriana in wild boars (Sus scrofa) from Latvia. Parasitol Res 119:3777–3783. https://doi.org/10.1007/s00436-020-06882-2
22. Rubiola S, Civera T, Ferroglio E, et al (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 and Waterborne Parasitology 18:e00074. https://doi.org/10.1016/j.fawpar.2020.e00074
23. Rubiola S, Civera T, Panebianco F, et al (2021) Molecular detection of cattle Sarcocystis spp. in North-West Italy highlights their association with bovine eosinophilic myositis. Parasites & Vectors 14:223. https://doi.org/10.1186/s13071-021-04722-5
24. Taylor MA, Boes J, Boireau P, Boué F, Claes M, Cook AJC, Dorny P, Enemark H, van der Giessen J, Hunt KR, Howell M, Kirjušina M, Nöckler K, Pozio E, Rossi P, Snow L, Theodoropoulos G, Vallée I, Vieira-Pinto MM, and Zimmer IA (2010): Development of harmonised schemes for the monitoring and reporting of Sarcocystis in animals and foodstuffs in the European Union. Supporting publications. EFSA-Q-2009-01074. https://www.efsa.europa.eu/en/supporting/pub/en-33
25. Vangeel L, Houf K, Chiers K, Vercruysse J, D'herde K and Ducatelle R (2007). Molecular-Based Identification of Sarcocystis hominis in Belgian Minced Beef. Journal of Food Protection, 70(6), 1523–1526. https://doi.org/10.4315/0362-028X-70.6.1523

Figures

Figure 1

Grossly visible lesions (arrowheads) detected at slaughter in different skeletal muscles and in the oesophagus (A-C); macroscopic cyst along the oesophageal muscular layers (D).

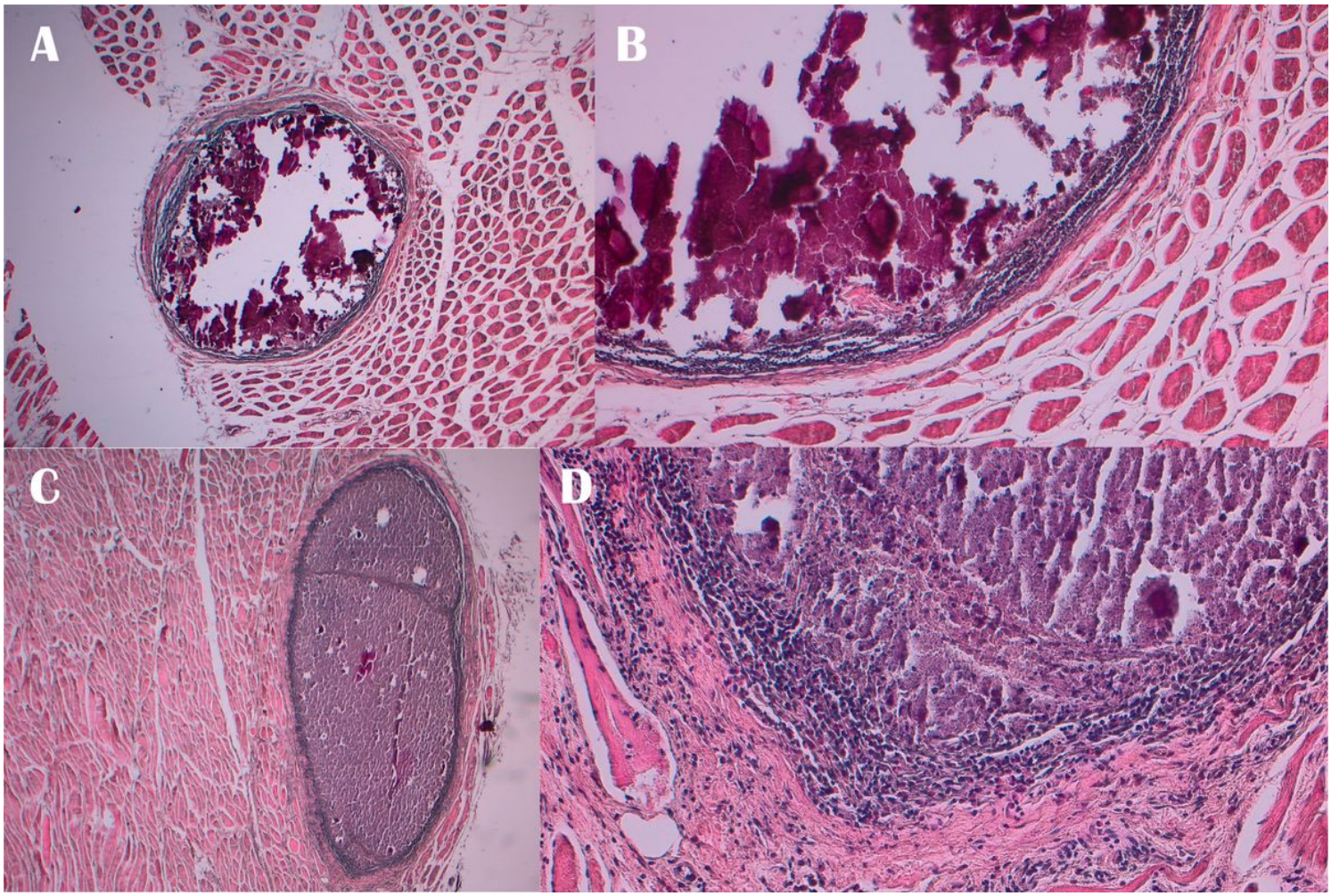

Figure 2

Microscopic appearance of the detected lesions. (A-B) Cross-section of the cyst with mineralized central area A: 25X; B: 100x. (C) Cross section of a cyst surrounded by a slight inflammation, fibrosis and atrophy of the muscle fibres; 25X. (D) Higher magnification of figure C showing the non-suppurative inflammatory infiltrate and fibrosis surrounding the cyst and the multifocal calcification in the core; 200x. Haematoxylin and eosin stain.

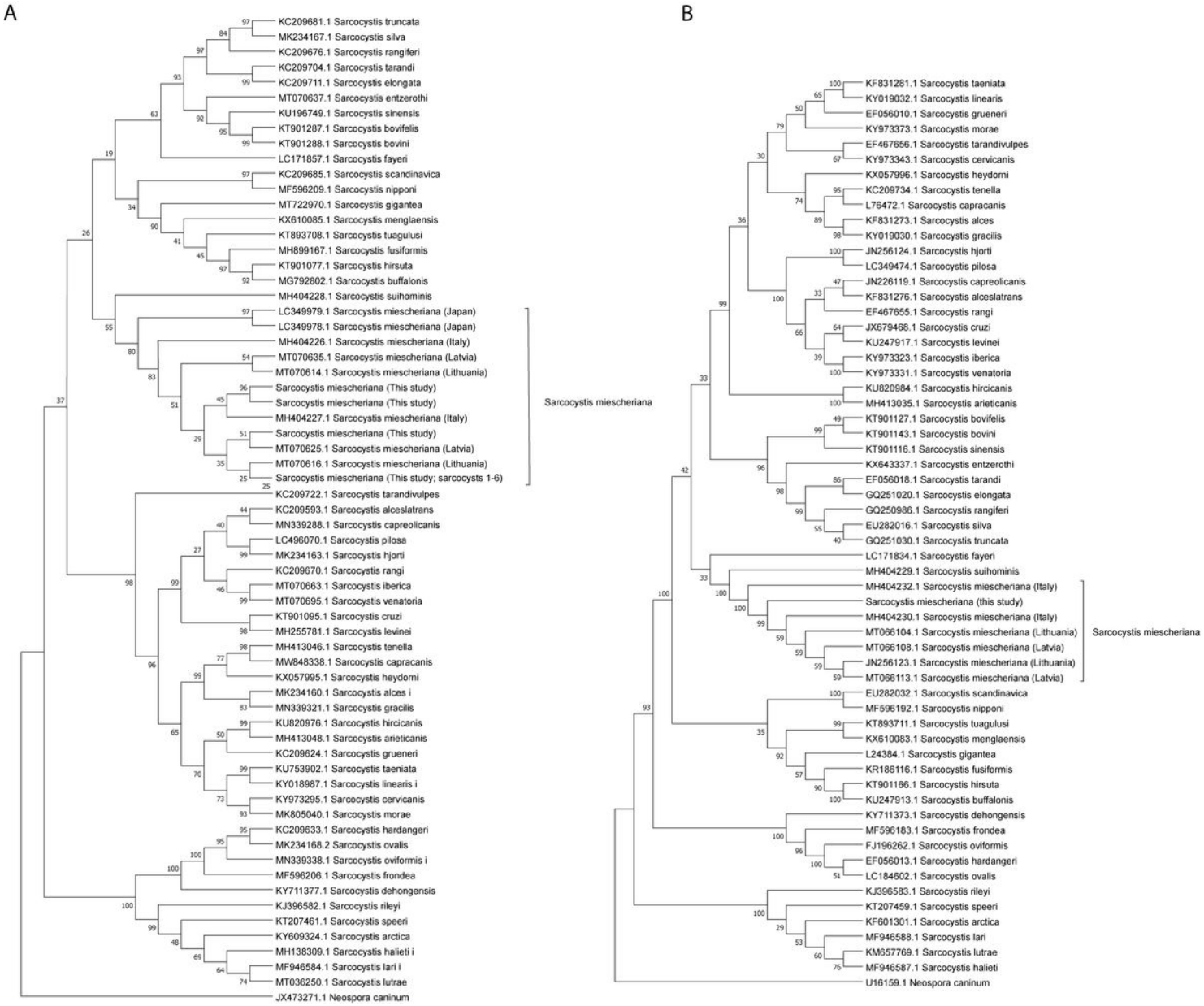

Figure 3

Phylogenetic trees for members of the *Sarcocystidae* family based on 70 partial sequences from 54 taxa of the *cox1*gene (a) and 60 sequences of the near-full-length 18S rRNA gene of 54 taxa (b), inferred using the neighbour-joining method. Six out of nine *cox1* generated sequences have been collapsed. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches (1000 replicates). GenBank accession no. of sequences are reported near the species names. Trees were rooted on *Neospora caninum*.