

Article

First Identification of Pathogenic and Zoonotic-Relevant *Sarcocystis hominis* and Other *Sarcocystis* Species in Slaughtered Cattle in Chile

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Simple Summary

Cattle can harbor muscle parasites of the genus *Sarcocystis* that are not detectable during routine slaughterhouse inspection but may have important implications for animal production and public health. Some of these parasites are zoonotic, meaning they can be transmitted from animals to humans through the consumption of raw or undercooked beef, causing intestinal disease. In Chile, information on the presence of these parasites in cattle destined for human consumption has been lacking. The aim of this study was to determine the occurrence and species composition of these parasites in slaughtered cattle from central Chile. Muscle samples from the heart and diaphragm of cattle were examined using microscopic and genetic methods. Although no parasites were visible to the naked eye, microscopic infection was common, particularly in the heart muscle. Importantly, this study provides the first evidence in Chile of a zoonotic parasite species in cattle, together with other species of veterinary importance. These results demonstrate that cattle in Chile participate in parasite transmission cycles involving animals and humans and highlight a potential food safety risk. These findings contribute to a better understanding of the occurrence of *Sarcocystis* spp. in cattle and underscore the value of ongoing monitoring to support food safety and animal health.



Academic Editor: Rodrigo Morchón García

Received: 13 January 2026

Revised: 14 February 2026

Accepted: 15 February 2026

Published: 24 February 2026

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Abstract

Sarcocystis species are apicomplexan protozoa infecting a wide range of domestic and wild animals, including cattle, in which several species are of zoonotic relevance. This study reports, for the first time, the detection and molecular identification of pathogenic and

zoonotic *Sarcocystis hominis* in slaughtered cattle from Central Chile. A total of 200 muscle samples (100 = myocardium, 100 = diaphragm) were examined by macroscopic inspection and tissue homogenization. Selected samples were additionally analyzed by histology, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and multiplex real-time PCR targeting the 18S rDNA. No macroscopic sarcocysts were observed, nonetheless microscopic sarcocysts were detected in 56% of assessed samples, with higher infection rates in the heart (91%) than in the diaphragm (21%). SEM and TEM analyses revealed thin-walled sarcocysts with finger-like protrusions in the diaphragm, as well as flattened hair-like projections in the myocardium. Molecular analysis identified *Sarcocystis cruzi* in all positive samples and detected additional DNA of *Sarcocystis bovifelis*/*Sarcocystis rommeli* and for the first time the zoonotic species *S. hominis*. These findings confirm the coexistence of canine-, feline-, and human-transmitted *Sarcocystis* species in Chilean cattle and highlight potential public health implications associated with consumption of raw or undercooked *S. hominis*-carrying beef meat. This constitutes the first molecular evidence of *S. hominis* in Chile, emphasizing the need for further surveillance and control measures in the meat production chain. These novel data on human *S. hominis* infections in Chile confirm the importance of initiating investigations on human sarcocystosis as this enteric parasitic disease is still sparsely considered by local public health authorities.

Keywords: *Sarcocystis cruzi*; *Sarcocystis hominis*; *Sarcocystis bovifelis*; cattle; zoonosis; molecular detection; Chile

1. Introduction

The genus *Sarcocystis* comprises some of the most prevalent protozoan parasites within superphylum Alveolata and is classified in the phylum Apicomplexa and is characterized by an obligatory two-host life cycle [1,2]. Herbivorous or omnivorous animals act as intermediate hosts, becoming infected through ingestion of water or food contaminated with sporocysts shed by definitive hosts, which are typically carnivores, omnivores or scavengers that acquire the infection by consuming infected tissues carrying *Sarcocystis* cysts (syn. sarcocysts) in the musculature [3–5]. More than 150 *Sarcocystis* species have been described in domestic and wild animals worldwide [6]. Current evidence indicates that cattle serve as intermediate hosts for at least seven recognized *Sarcocystis* species including *Sarcocystis cruzi*, *Sarcocystis rommeli* (formerly known as *Sarcocystis sinensis*-like), *Sarcocystis hirsuta*, *Sarcocystis hominis*, *Sarcocystis heydorni*, *Sarcocystis bovifelis*, *Sarcocystis bovini* and the recently described species *Sarcocystis sigmoideus* [7–9]. Canids act as definitive hosts for the species *S. cruzi* (thin-walled cysts), whereas felids are definitive hosts for the species *S. hirsuta*, *S. rommeli*, *S. bovifelis* and presumably *S. bovini* (all thick-walled cysts) [9]. Moreover, humans are considered definitive hosts for *S. hominis* (thick-walled cysts), *S. heydorni* (thin-walled cysts) and *S. sigmoideus* (thick-walled cysts) [8–11]. Among them, *S. cruzi* is the most prevalent species affecting cattle throughout the world and the most pathogenic one [12,13], resulting in acute disease that can cause abortion and mortality, thereby generating substantial economic losses for the cattle industry [14–17], depending on the specific isolate and the quantity of sporocysts consumed among other factors [16–19]. The cardiac muscle is the tissue most affected by *S. cruzi* [20,21] and other bovine tissues rich in striated muscle, such as the esophagus and skeletal musculature, are more associated with infections by *S. hirsuta* and *S. hominis* [22]. *Sarcocystis hominis* and *S. heydorni* are of zoonotic relevance, with humans acting as definitive hosts; similarly, *S. sigmoideus* has also been recognized as a zoonotic species [1,2]. Additionally, bovine sarcocystosis has been linked to an inflammatory condition of striated muscles termed bovine eosinophilic

myositis (BEM) which is detected during meat inspection [17,23–25]. Here, several species of *Sarcocystis* have been associated with BEM such as *S. hominis*, [17,23] as well as infections by *S. bovifelis*, *S. cruzi*, *S. hirsuta* and *S. sigmoideus* [24].

Diagnosis of bovine sarcocystosis is primarily based on homogenization and artificial digestion of meat samples, histopathological evaluation of stained tissues, and examination of fresh specimens [9,24,26], jointly with analyses of cyst wall morphology by light or electron microscopy [13,27,28]. Morphological characteristics such as sarcocyst size, wall thickness, wall morphology, and septation aid in distinguishing species, but fresh samples with abundant cysts are required for accurate final diagnosis [5,17,21]. Molecular tools, particularly sequencing of the 18S rRNA gene, now provide a more sensitive and reliable alternative for species identification [29].

The global prevalence of *Sarcocystis* spp. in bovine musculature can reach nearly 100% in adult animals worldwide [13,25]. However, *S. hirsuta* (originating from felids) is the species most frequently reported to produce macroscopic sarcocysts in cattle which can be detected during carcass inspection [17]. Studies analyzing minced meat destined for human consumption in Europe and Asia have revealed the presence of zoonotic *S. hominis* in these samples [30–32]. This raises concerns about the underestimated risk to public health, especially as consumption of raw minced beef is common in many countries worldwide [9]. Human infection, referred to as sarcocystosis, may present with symptoms such as loss of appetite, nausea, vomiting, diarrhea, abdominal pain, respiratory complications, and tachycardia [2,3,17].

Despite their wide distribution and veterinary relevance, studies on *Sarcocystis* in cattle meat remain limited in South America. To date, *Sarcocystis* spp. infections in cattle have been documented in Argentina with *S. cruzi*, *S. hirsuta* and *S. hominis* being identified [26]. In Uruguay, *S. cruzi* has been confirmed in bovines associated with myocardial lesions [33]. In Peru, *Sarcocystis* spp. were reported in cattle from slaughterhouses, suggesting a high regional prevalence [34]. In Brazil, the occurrence of *S. cruzi* and *S. hominis* has also been described [35,36], indicating that multiple *Sarcocystis* species are widely distributed among South American cattle populations.

Currently, no published data exists on the presence of *Sarcocystis* species in cattle from Chile. Therefore, the main objective of this study was to determine, the occurrence of bovine sarcocystosis and to characterize the species spectrum through molecular analyses in slaughtered cattle intended for human consumption in Chile.

2. Materials and Methods

2.1. Bovine Samples

A total of 200 muscle samples were collected from 100 cattle obtained in a slaughterhouse under federal inspection located in Maule Region, Central Chile, which receives cattle from several locations of different regions of Chile besides Maule such as Valparaíso, Ñuble, Bío Bío, Araucanía, Los Ríos and Aysen. The sampling design was consistent with previous studies investigating *Sarcocystis* spp. in slaughtered cattle using comparable epidemiological and molecular approaches [8]. Samples were obtained in three samplings during the first semester of 2024. The samples (approximately 100 g per tissue) were derived from the following bovine tissues: 100 from myocardium (left ventricle) and 100 from diaphragm (diaphragmatic pillar), totalizing 200 tissue fragments. Sampled cattle showed no clinical signs compatible with infectious diseases prior to slaughter. The tissues were individually packaged in plastic bags and transported in polystyrene boxes with ice packs until their arrival at the Laboratory of Veterinary Parasitology of Santo Tomás University in Maule Region, Talca, where the tissues were stored at 4 °C until analysis. Examination of animal tissues was performed within 96 h post-collection.

2.2. Macroscopic Examination

All 200 muscle tissue samples were examined by the naked eye to detect either macroscopic visible sarcocysts or indications of *Sarcocystis*-mediated myositis and/or fibrosis. Additionally, transversal cuts were performed in each muscle sample to detect intramuscular cysts of *Sarcocystis* spp.

2.3. Tissue Homogenization and Light Microscopy Analysis

For tissue homogenization, the protocol described by Moré et al. [26] and Rubiola et al. [24] was applied with slight modifications. Briefly, ten grams of each muscle tissue sample was placed in a sterile 50 mL tube (Falcon) and minced with up to 50 mL of sterile phosphate-buffered saline (PBS) using a tissue homogenizer, filtered with a 300- μ m-pore-sized sieve, and centrifuged at $600\times g$ for 5 min. The supernatant was discarded, and the resulting pellet was resuspended in 20 mL of sterile PBS, transferred into a Petri dish and observed using an inverted microscope coupled with a camera (ICOE IV5100FL Ningbo Icoe Commodity Co., Ltd., Ningbo, China) at $10\times$ and $40\times$ magnifications. Sarcocysts were detected under light microscopy, classified as thick- or thin-walled, and measured in length and width by using ImageView[®] version x64 software (Leica Microsystems GmbH, Wetzlar, Germany). In addition, samples from the myocardium and diaphragm were classified according to the observed number of sarcocysts as follows: low positive (fewer than 5 cysts) or moderate-to-high positive (more than 5 cysts) according to Moré et al. [37]. Individual sarcocysts from moderate-to-high-positive samples were recovered by using Pasteur pipettes into 2 mL Eppendorf tubes, making a total of 18 sarcocyst pooled samples (9 obtained from the myocardium and 9 from the diaphragm). One third (3 samples from each anatomical location) were transferred into 1.5 mL nuclease-free tubes (Eppendorf, Hamburg, Germany) and preserved at -20°C for further molecular analysis. And two thirds (6 samples from each anatomical location) were fixed in 2.5% glutaraldehyde for 2 h, washed 3 times with sterile PBS and stored for further SEM and analyses. Histological examination was restricted to tissues containing more than 15 sarcocysts to ensure adequate parasite representation for morphological evaluation.

2.4. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) Analyses

Isolated sarcocysts were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide (Merck, Darmstadt, Germany), washed in distilled water, dehydrated, critical point dried by CO_2 treatment and sprayed with gold. All samples were examined with a Philips XL30 scanning electron microscope at the Institute of Anatomy and Cell Biology of the Justus Liebig University Giessen, Germany. Sarcocyst wall ultrastructure was evaluated as previously described by Moré et al. [26].

For TEM analysis, isolated sarcocysts were fixed in 2.5% glutaraldehyde and post-fixed in buffer containing 1% osmium tetroxide (Merck, Darmstadt, Germany). After thoroughly washing in distilled water, the samples were incubated overnight in 2% aqueous uranyl acetate (Merck, Darmstadt, Germany) at 4°C , dehydrated in ethanol and embedded in Epon (all Merck, Darmstadt, Germany). Ultrathin sections of the cured blocks were mounted on formvar-coated grids and stained with uranyl acetate and Reynolds lead citrate (both Merck, Darmstadt, Germany). Ultrathin sections were analyzed using a transmission electron microscope (Zeiss EM 900EL, Zeiss, Oberkochen, Germany) equipped with a slow scan 2 K CCD camera (TRS, Tröndle, Moorenweis, Germany).

2.5. Histological Examination

For the histological analysis, all tissue samples showing more than 15 sarcocysts detected by light microscopy using the homogenization method were gathered, and from these, 10 samples from each anatomical location were randomly selected for histological

examination. Tissue samples were fixed in 10% buffered formalin to pause autolysis. After fixation, each sample was trimmed as appropriate and embedded in paraffin. The paraffin blocks were stored at room temperature (RT) until sectioned on a microtome to yield 5-µm-thick sections. These were stained with hematoxylin and eosin (H&E) by using conventional methods. After staining, sections were microscopically examined for detection of sarcocysts in tissue by using a light microscope coupled with a camera (Olympus CX23, Olympus Corporation, Tokyo, Japan) at ×400 magnification [38].

2.6. Molecular Characterization via Real-Time PCR

Samples with high parasitic (sarcocyst) loads identified by light microscopy via the homogenization technique were used for molecular analysis. Here, *Sarcocystis*-cyst DNA was extracted using the DNeasy Blood & Tissue Kit[®] (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The routine procedure of DNA extraction included a negative processing control. The detection and differentiation of *Sarcocystis* spp. was performed applying the multiplex PCR method described by Moré et al. [36] targeting the nuclear small subunit (18S) rDNA genes of *S. cruzi*, *S. hominis*, *S. hirsuta* and *S. bovifelis/S. rommeli* as shown in Table 1. This analysis has shown sufficient variability for the discrimination of *Sarcocystis* spp. found in cattle [36]. All reactions were conducted with a negative control (0.5% TE buffer as sample) and a no-template control (NTC) according to [37]. In addition, as positive control a mix of plasmids containing target genes was used as previously described [37]. Optimal cycling conditions were 95 °C (4 min) followed by 40 cycles of 95 °C (15 s), 62 °C (40 s). Optimized reaction mixtures for the BovSarcoMultiplex real-time PCR were: total volume of 20 µL, containing 2.5 µL of template DNA and 17.5 µL of SensiFAST[™] Probe NO-ROX Kit (Bioline Meridian Lifescience, Memphis, TN, USA), with a final concentration of each primer and the following probes: SarcoRTF (600 nM), SarcoRTR (400 nM) and ShirsutaRTR (300 nM), Shirsuta-Probe (200 nM), Scruzi-Probe (100 nM), Shom-LNA-Probe (200 nM) and Ssin-LNA-Probe (300 nM) following previous studies [37].

Table 1. Primers and probes designed to identify *Sarcocystis* spp. from cattle by real-time PCR according to Moré et al. [37].

Type	Name	5'–3' Sequence with Modifications (in Brackets)
Forward Primer	SarcoRTF	TCTGCTGGAAGCAATCAGTC
Reverse Primer	SarcoRTR	AGGCAATAAGCCTCTTCAA
Reverse Primer	ShirsutaRTR	GCAACAATAAGCCTCTTCAAA
CDL Probe	Shirsuta	(FAM)-CCTTCTAATGAGGGTGTGTACTTGATGAA-(BHQ1)
CDL Probe	Scruzi	(RED)-ACCCATCTATATTGGGATAATACCATTACT-(BHQ1)
LNA [™] Probe	Shom-LNA	(Cy5)-TCT(+T)A(+A)TA(+T)AA(+T)GA(+T)TG(+A)(+T)TG(+A)(+T)TGA-(BHQ2)
LNA [™] Probe	Ssin-LNA	(HEX)-CTG(+A)TG(+A)CT(+T)TC(+A)GT(+A)GTCAT-(BHQ1)

Reference: CDL = conventional dual labeled; LNA = locked nucleic acids modification; bases marked as (+A) or (+T) indicates an LNA modification. BHQ = Black Hole Quencher. Fluorophores: Cy5 = Cyanine dye 5; FAM = 6-carboxyfluorescein; HEX = hexachloro-6-carboxyfluorescein; RED = Texas Red.

3. Results

3.1. Macroscopic Evaluation

No cystic structures or macroscopic lesions were observed by the naked eye in 200 tissue fragments, neither from the myocardium nor from the diaphragm.

3.2. Microscopic Examination

Tissue sarcocysts were observed in 112 of the 200 muscle samples analyzed (56%). The highest frequency of infection was found in cardiac muscle (91%; 91/100) followed by diaphragmatic muscle (21%; 21/100).

The sarcocysts detected in myocardial tissue under the homogenization technique were mostly observed extracellularly of muscle fibers, containing a thin cyst wall (Figure 1A–C) and internal septa (Figure 1A–C). These features were also observed in the same muscle samples stained with H&E, presenting variable sizes and shapes, including elongated, elliptical and globular sarcocysts (Figure 1A–C). Morphometric measurements indicated that myocardial cysts averaged 360.2 μm in length and 110.3 μm in width, with thin capsules measuring approximately 1.3 μm (Figure 1A–C), compatible with *S. cruzi*. In contrast, after processing by the tissue homogenization technique, the cysts present in the diaphragmatic muscle were intracellular, exhibiting a capsule or enclosing wall (Figure 1D–F) and well-defined internal septa (Figure 1D–F) containing bradyzoites (Figure 1F). By using both homogenization and histological techniques, tissue cysts from the diaphragm resulted in different sizes and shapes, adapting to the muscle fibers. Here, cysts averaged 269.6 μm in length and 82.6 μm in width, being thin-walled but also displaying thicker capsules measuring approximately 4.28 μm (Figure 1D–F) compatible with *S. hominis*, among others, and *S. cruzi*. In addition, larger cysts were also identified, located extracellularly and with an extremely thin capsule ($<1 \mu\text{m}$), which could indicate structural variability attributable to the species or developmental stage of the parasites (Figure 1C). Neither inflammation nor any other microscopic lesions (e.g., granuloma, fibrosis) were observed around identified sarcocysts, although muscle fibers were increased in size due to the presence of the parasites.

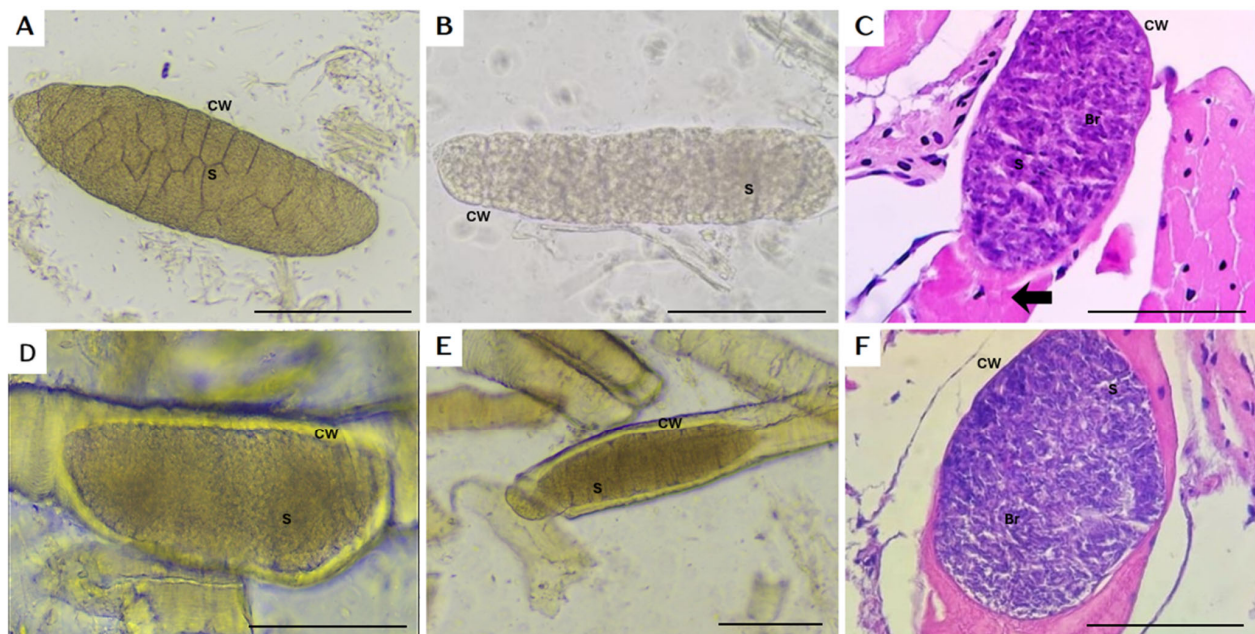


Figure 1. Morphology of *Sarcocystis* spp. tissue cysts from cardiac and diaphragmatic muscle of slaughtered cattle observed by light microscopy and H&E staining. (A) Tissue cyst in myocardium located outside the muscle fibers, of 530.2 μm length, 164.3 μm width, and with a wall thickness of 1.03 μm (100 $\times$) with several internal septa that form compartments containing bradyzoites. (B) Tissue cyst in myocardium located outside the muscle fibers (40 $\times$) displaying hair-like protrusions. (C) Section of myocardium containing an elliptical thin-walled tissue cyst (100 $\times$). The arrow indicates hypertrophy of the muscle fiber associated with the localization of the tissue cyst. (D) Tissue cyst in diaphragm inside the muscle fiber, of 236.37 μm length and 90.55 μm width, with a thick cyst wall of 4.63 μm (100 $\times$). (E) Tissue cyst in diaphragm within the muscle fiber, measuring 317.38 μm length and 85.78 μm width, with a thick capsule of 4.72 μm (40 $\times$). (F) Section of diaphragm stained with H&E containing a globular thin-walled tissue cyst (100 $\times$). CW: cyst wall; S: septa; Br: bradyzoites. (A,B,D,E): analyzed via homogenization technique; (C,D): analyzed by histology and H&E staining. Scale Bar (A): 200 μm ; (B–D,F): 100 μm ; (E): 150 μm .

3.3. SEM and TEM Analyses

SEM analysis of isolated bovine sarcocysts from the diaphragm and myocardium revealed whole cysts exhibiting rounded anterior and posterior ends (Figure 2A,E), with cell wall surfaces varying in their ultrastructure, showing striated finger-like protrusions folded over the surface (Figure 2B,C) and flattened hair-like protrusions (Figure 2F,G) in diaphragm muscle and myocardium samples respectively, being compatible with the outer morphology of *S. hominis* and *S. cruzi* respectively. The cyst displaying finger-shaped protrusions was assigned to *S. hominis* based on the concordance between its characteristic wall morphology and the molecular results; however, given the known morphological overlap among *Sarcocystis* spp., this interpretation should be made cautiously, as similar features have occasionally been reported in closely related species such as *S. bovifelis*. TEM analysis displayed thin-walled sarcocysts with fine cyst wall protrusions on the sarcocyst wall (Figure 2D) and packets of bradyzoites (Figure 2H) in the myocardium.

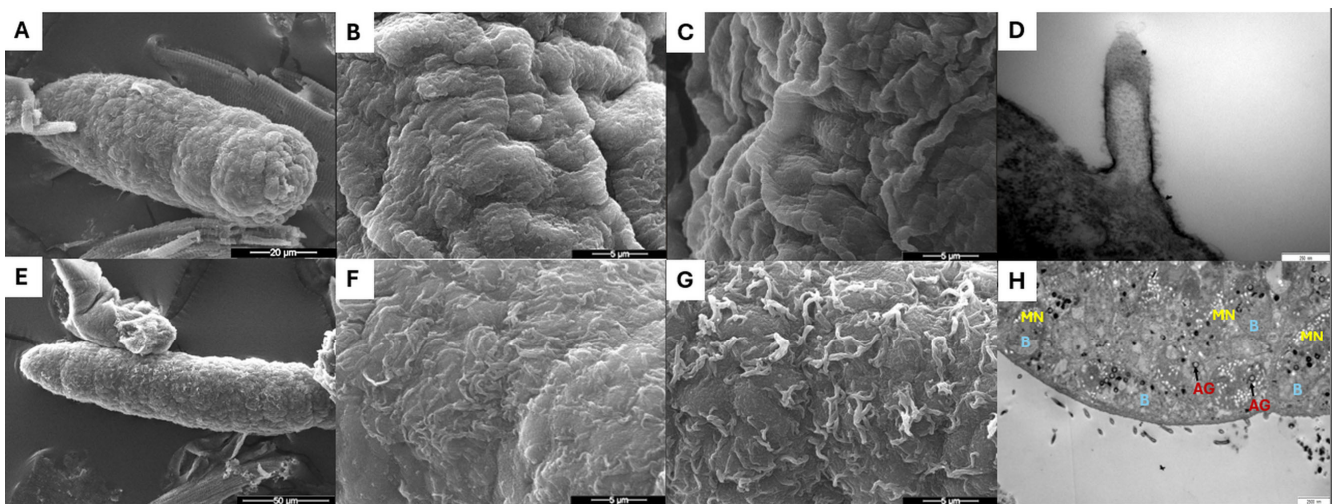


Figure 2. Morphology of isolated *Sarcocystis* spp. tissue cysts from diaphragmatic muscle (A–C) and myocardium (D–H) of slaughtered cattle observed by scanning electron microscopy (SEM) (A–C,E–G) and transmission electron microscopy (TEM) (D,H). (A) SEM image of isolated *Sarcocystis* sp. cyst showing elliptic shape and rounded ends on both sides of the cyst. (B,C) Surface of cyst wall showing finger-like protrusions. (D) TEM image of a single fine cyst wall protrusion on the sarcocyst wall. TEM Bar = 250 nm. (E) Tissue cyst showing elongated shape and rounded ends. (F,G) Surface of tissue cyst from myocardium displaying flattened hair-like protrusions. (H) TEM image of thin-walled sarcocyst with packets of bradyzoites (B), amylopectin granules (AG); and micronemes (MN).

3.4. Molecular *Sarcocystis* Species Identification

Molecular analysis of the parasites was performed using multiplex real-time PCR analysis targeting the nuclear small subunit (18S) rDNA genes of *S. cruzi*, *S. hominis*, *S. sinensis*, *S. hirsuta* and *S. bovifelis*. Cycle threshold (Ct) values were interpreted as indicators of parasite DNA load, with Ct values < 25 considered high, 25–30 moderate, and >30 low DNA amounts. As a result, all six samples tested were positive for *S. cruzi* with low Ct values (moderate to high amounts of DNA in the samples). In addition, one sarcocyst pooled sample from the diaphragm also showed signals, with higher Ct values (lower amounts of DNA) for *S. bovifelis*/*S. rommeli* and *S. hominis*, respectively.

4. Discussion

From the veterinary perspective, certain *Sarcocystis* species have been reported as being pathogenic for bovines, inflicting acute bovine sarcocystosis and leading to abortion and death [14–16] and causing carcass condemnation in most cases, and accompanied by

eosinophilic myositis [12,24], resulting in all these cases in significant economic losses for the livestock industry [9]. Cases of human sarcocystosis have been reported following the consumption of either raw or undercooked beef meat containing either *S. hominis* or *S. heydorni* sarcocysts, with both being the main source of human infection. Diagnosis of *Sarcocystis* in cattle is mainly premised on examination of fresh muscle tissue samples, homogenization and artificial digestion of meat specimens for further light microscopy analysis, histopathology of bovine tissue with H&E staining, SEM/TEM techniques, and molecular analyses [9,37,39].

Our results show the presence of *Sarcocystis* DNA from the species *S. cruzi*, *S. bovifelis*/*S. rommeli* and the zoonotic-relevant species *S. hominis*, being the first report of this species in cattle beef destined for human consumption in Chile. In addition, the occurrences of *S. bovifelis*/*S. rommeli* along with *S. cruzi* confirm the close interaction of cattle with dogs (canids) and cats (felids), which act as definitive hosts of these parasites, along with humans in the case of *S. hominis* [26].

Prevalence of *S. hominis* infection in cattle is variable in different parts of the world, with the esophagus and other muscles, but not the myocardium, being the most affected [26]. Therefore, the prevalence and abundance of *Sarcocystis* species depend on the type of muscle analyzed, with muscles from the heart and diaphragm being the target colonizing areas of *Sarcocystis* spp. in cattle [1]. In the present study, a significantly higher occurrence rate of *Sarcocystis* spp. cysts was observed in the heart (91%) than in the diaphragm (21%). Accordingly, high occurrence rates of *Sarcocystis* spp. have been reported in hearts, ranging between 58% and 99.5%, and with a lower magnitude in diaphragms (around 58%) from cattle in different countries, such as Iran [40,41], Italy [42], the Netherlands [43], Brazil [35] and Argentina [26].

Here, the predominance of *S. cruzi* in both locations was confirmed by real-time PCR based on the amount of DNA of this species in analyzed samples. Meanwhile, *S. hominis* was found in the diaphragm along with *S. bovifelis*/*S. rommeli* and *S. cruzi* according to other studies in bovines including *S. bovini* [44–47]. A limitation of the present study is that the real-time PCR used targeting the 18S rDNA was not designed to detect *S. heydorni* and *S. sigmoideus* and, consequently, their presence may have gone undetected. Intriguingly, no visible macrocysts were detected during macroscopic inspection. This feature has also been described in other reports of *S. hominis* and other large sarcocysts in cattle [48–50]. Moreover, as mentioned, in the myocardium the presence of *S. cruzi* was observed in a higher frequency than in the diaphragm, and this finding was also confirmed via light and SEM microscopy analyses and via histological techniques. Moreover, for the species *S. bovifelis*/*S. rommeli* their presence was only reported via molecular analyses, suggesting that, as stated in other studies, the isolation of individual sarcocysts may be ineffective when the prevalence of infection of certain species is low [44]. Furthermore, our result supports reports showing that cattle are substantially prone to a relatively high prevalence of *Sarcocystis* infections and therefore play a critical role in dissemination of zoonotic and non-zoonotic species to humans, domestic dogs and cats, respectively. Moreover, it is demonstrated that *S. cruzi* and *S. hominis* are the two most prevalent species isolated from cattle, globally, which is relevant to veterinary and public health [9].

Overall, analyses on minced meat have detected zoonotic species of *Sarcocystis* [30]. However, those results are difficult to compare since minced meat is composed of several organs of different carcasses. Based in our results, DNA from *S. hominis* has been found in the diaphragm of cattle destined for human consumption in Chile, therefore, as stated elsewhere, when *S. hominis*- and/or *S. heydorni*-infected diaphragms enter the food chain, they might pose a high risk for public health, depending on their further processing [46] and representing a potential food safety issue. Therefore, we call for further research in this

line as well as to follow hygienic protocols, especially avoiding the consumption of raw or undercooked beef in order to reduce the risk of *Sarcocystis* infection in humans.

5. Conclusions

This study provides the first molecular evidence of *S. cruzi*, *S. bovifelis*/*S. rommeli*, and the zoonotic *S. hominis* in cattle from Chile, revealing a high infection frequency and the coexistence of multiple host–parasite cycles involving canids, felids, and humans. The predominance of *S. cruzi* in cardiac muscle and detection of *S. hominis* in the diaphragm emphasize the potential tissue preference of these species. The absence of macroscopic cysts in muscles reinforces the need for molecular surveillance to detect potential zoonotic *Sarcocystis* infections. It also should be considered that the absence of macroscopic cysts in the examined tissues should be interpreted with caution, as these structures may occur more commonly in other organs with a high proportion of striated muscle, such as the esophagus, which was not evaluated in the present study. These findings are consistent with previous reports describing a high occurrence of *Sarcocystis* infections in slaughtered cattle across different geographic regions [8,19,26,35] and highlight potential food safety concerns and the importance of implementing control measures to prevent zoonotic transmission through bovine meat consumption.

Author Contributions: Conceptualization, T.M.-C., M.J.T.F., E.P.S., C.A.G., A.H., F.F.S., F.Z., P.H.H., C.H., A.T., U.G., W.B. and G.M.; Methodology and formal analysis, T.M.-C., A.H., P.H.H., U.G., C.H., A.T., U.G., W.B. and G.M.; Methodology, T.M.-C., M.J.T.F., E.P.S., W.B. and G.M.; Validation, T.M.-C., A.H., F.F.S., F.Z., P.H.H., C.H., A.T., W.B. and G.M.; Writing—review and editing, T.M.-C., M.J.T.F., E.P.S., A.H., F.F.S., F.Z., P.H.H., U.G., C.H., A.T., W.B. and G.M.; Writing—original draft preparation, T.M.-C., M.J.T.F., E.P.S., A.H. and P.H.H.; Data curation, T.M.-C., M.J.T.F., E.P.S., C.A.G., A.H., F.F.S., F.Z., P.H.H., C.H., W.B., U.G. and G.M.; Investigation and project administration, T.M.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: All animal procedures were performed in accordance with the current Chilean Animal Protection Laws (Law No. 20.380 on animal protection and Decree No. 28 on animal welfare).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: We would like to acknowledge Anika Seipp (Institute of Anatomy and Cell Biology, Faculty of Human Medicine, Justus Liebig University Giessen) for her excellent technical support for generating SEM and TEM images.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy

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