Ultrastructural and molecular identification of *Sarcocystis tenella* (Protozoa, Apicomplexa) in naturally infected Korean native goats

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ABSTRACT: Sheep is the intermediate host of the protozoan parasite *Sarcocystis tenella*, while the dog is its definitive host. This study was conducted to determine the prevalence of natural infection with *S. tenella* in slaughtered Korean native goat, *Capra hircus coreanae,* in the Republic of Korea. H-E stained heart tissues were investigated for the presence of sarcocysts. Of the 103 goats, three (2.91%) were diagnosed as positive for *S. tenella* by light, electron microscopic and molecular examination. The histopathological study showed a low frequency of microscopic *Sarcocystis* infection in slaughtered goats. In transmission electron microscopy, the sarcocysts were confirmed as *S. tenella.* Further DNA sequencing and phylogenic analysis support our identification of *S. tenella* with a 18S rRNA sequence identity of 100% between the experimental sequence and *S. tenella*. To our knowledge, this is the first record of *S. tenella* in Korean native goats from Korea. We thus report that the domestic goat is another intermediate host for *S. tenella.*

Keywords: *Capra hircus coreanae*; cardiac muscle; *Sarcocystis tenella*; sarcocysts; bradyzoites; ultrastructure; molecular biology

   Sarcocystis species are common parasites with a wide range of hosts and are obligatory intracellular protozoa (Dubey et al. 1989). Also, *Sarcocystis* is one of the most prevalent protozoan parasites in ruminant livestock such as cattle, sheep and goat. Ruminant intermediate hosts become infected by ingesting infective sporocysts from the faeces of infected carnivores (Dubey et al. 1989). A comprehensive cataloguing of *Sarcocystis* spp. (Odening 1998) indicates that 189 species of *Sarcocystis* have been identified to date; however, both definitive and intermediate hosts are known for only 46% of these species. There are three reported species of *Sarcocystis* in domestic goats: *S. capracanis, S. hirci-*

canis and *S. caprafelis* (syn. *S. moulei*). *S. capracanis* and *S. hircicanis* produce microscopic sarcocysts while *S. caprafelis* produces macroscopic cysts. *S. capracanis* is the most pathogenic species in goats, causing fever, weakness, anorexia, weight loss, tremors, irritability, abortion and death (Dubey et al. 1989). Sheep are intermediate hosts of four *Sarcocystis* species. *S. gigantea* (syn. *S. ovifelis*) and *S. medusiformis* lead to the development of macroscopic cysts, *S. tenella* (syn. *S. ovicanis*) and *S. arieticanis* lead to the development of microscopically visible cysts in sheep muscles (Collins et al. 1979; O'Donoghue and Ford 1986; O'Donoghue et al. 1996).

To our knowledge, there is no report available as yet of *S. tenella* in goats. In this study, we report the natural infection of *S. tenella* in Korean native goat for the first time. The ultrastructure as well as DNA sequence of goat samples was also studied.

MATERIAL AND METHODS

Sample collection and light microscopy. The 103 cardiac muscle samples of Korean native goats, *Capra hircus coreanae*, were obtained from January to August, 2014 in a slaughterhouse located in Wonju city, the Republic of Korea. None of these goats presented any neurological or respiratory signs at that moment. A portion of each cardiac muscle sample was fixed in 10% neutral buffered formaldehyde, embedded in paraffin, sectioned at 4 μm, and stained with haematoxylin and eosin for histological examination. The rest of the samples were stored at −20 °C for PCR.

Transmission electron microscopy (TEM). The paraffin block of heart muscle containing cysts identified by light microscopy was deparaffinised with xylene, and fixed with 2.5% glutaraldehyde solution in 0.1M phosphate buffer (pH 7.4) for 4 h. After washing with buffer, the specimens were post-fixed with 1% osmium tetroxide at 4 °C for 4 h. Afterwards, the specimens were dehydrated in a graded ethyl alcohol series and two changes of propylene oxide, and embedded in epon mixture. The ultrathin sections were stained with uranyl acetate and lead citrate and examined using a Tecnai G2 transmission electron microscope at 120 kV (FEI, Oregon, USA).

DNA extraction and amplification. Genomic DNA was extracted from *Sarcocystis* specimens using a DNeasy® Blood and tissue kit (Qiagen, Alameda, CA) according to the manufacturer's instructions. The

Sarcocystis-infected cardiac muscle was homogenised with lysis buffer and incubated with proteinase K at 56 °C. Then, neutralisation buffer and ethanol was added and the samples were transferred into DNeasy® columns. The genomic DNA was eluted with TE (tris-EDTA) buffer. Several sequences from *S. tenella*, *S. capracanis*, *S. alces*, *S. hjorti*, and *S. tarandivulpes* were aligned using multiple sequence alignment (Clustal Omega, http://www.ebi.ac.uk/Tools/msa/clustalo), and then common *Sarcocystis* primers were designed using an online tool (Primer3Plus, http://www.bioinformatics.nl/cgi-bin/primer3plus/primer-3plus.cgi). An 18S rRNA region spanning the internal transcribed spacer 2 (ITS2) gene (DNA) was amplified to obtain genomic DNA sequence using polymerase chain reaction (PCR). The oligonucleotide sequences of primers employed to detect the Gordius 18S rRNA region spanning the ITS2 gene (DNA), were 5'-GAATCCAAACCCCTTTCAGAGT-3' (forward) and 5'-AGAATTTCACCTCTG-3' (reverse). The primer set was designed to yield a 1448 bp product, variable for all sequences aligned. PCR reactions were performed in MyCycler Personal Thermal Cycler (Bio-Rad Laboratories, Hercules, CA) using EmeraldAmp GT PCR Master Mix (Takara, Shiga, Japan) with 1 μl DNA. Cycling conditions were the following: 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min. The PCR products were next visualised using electrophoresis on 1.2% agarose gels, and then purified using the QIAquick PCR purification kit (Qiagen, Alameda, CA).

Sequence analysis and phylogenetic analysis. PCR amplicons were directly sequenced using ABI Prism Big Dye terminator v. 3.0 ready reaction cycle sequencing kits (Applied Biosystems) with the same primers as those used in PCR. The sequencing reactions were run on a PE Applied Biosystems 3100 automated sequencer. The sequence data were aligned using the Clustal Omega program (clustal O 1.2.1). A phylogenetic tree based on sequence analysis was constructed by neighbour-joining using blast tree (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome).

RESULTS

Cysts of *S. tenella* were found in H-E stained cardiac tissue samples of Korean native goats. Histopathological examination of heart samples revealed sarcocysts in three out of 103 goats (2.91%).
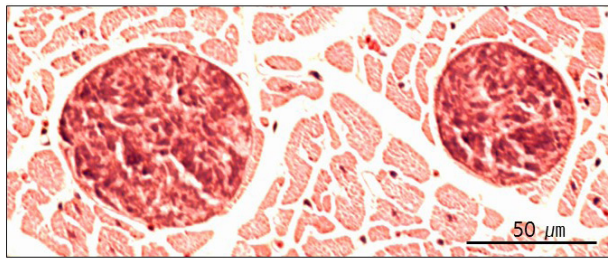
Figure 1. Photograph of *Sarcocystis* from the H-E stained heart tissue of a Korean native goat infected with *S. tenella*. The infection appears as round cysts embedded in the muscle fibres of the host cells

The cysts were always within a muscle fibre. The sarcocysts consisting of parasites in different stages of their development were abundant in infected cardiac muscle. No inflammatory reaction such as severe eosinophilic myositis or diffuse leukocytic infiltration was seen in tissue surrounding the sarcocysts. The sarcoplasm of the myocardium presented with numerous bradyzoites enclosed in the thin smooth host cell wall, but the wall of sarcocysts was not discernible. Cyst morphology ranged from oval to spherical and cysts measured 30–65 × 52–221 (mean 53.5 × 117.0) µm in size (Figure 1). However, specific species could not be accurately described using histological analysis. The difference in morphology could not be observed between the corresponding sarcocysts from different host species. Images from TEM show a relatively sim-

ple and thin sarcocyst wall (maximal thickness approximately 1.6 µm) with short, irregular, villous protrusions (Figure 2).

The walls of cysts were characterised by thickening of the inner dense layer and the presence of numerous small pits. The membrane of these pits had no thickening of the inner dense layer and the pits themselves formed a neck resembling the micropinocytic pits or caveolae of the cells of multicellular organisms (Figures 2 and 5). The electron-dense layer underlying the parasitophorous vacuolar membrane was interrupted at intervals, especially in areas at the base of, or between, villi. The ground substance was devoid of microtubules, even as it formed villous protrusions, and was continuous with the septa that run between bradyzoites and metrocytes (Figures 3–7). The villous protrusions without microtubules were round, quadrate or flattened (Figures 2 and 5). Bradyzoites measured approximately 6–8 × 1.5–3 µm.

Bradyzoites were surrounded by two (inner and outer) membranes, forming the pellicle, and had distinct anterior and posterior ends (Figures 4 and 8). The apical complex, numerous micronemes and up to 12 rhoptries were located at the anterior region (Figures 3 and 4). Inclusion bodies, including amylopectin, lipid, and electron-dense granules, were found in the central region of the bradyzoites, and cell organelles, including the nucleus, were confined primarily to the posterior regions. The
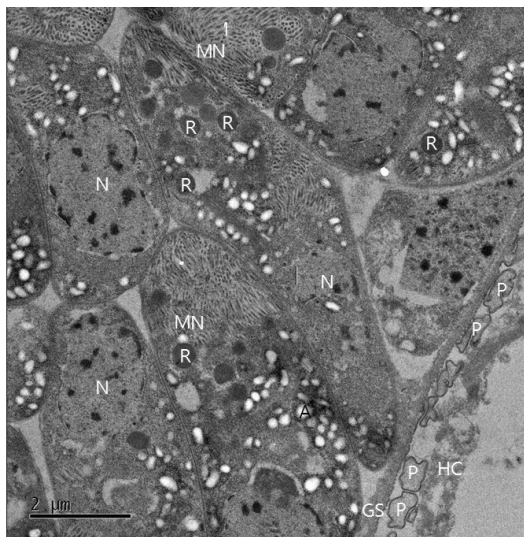

Figure 2. SEM of tissue cyst of *Sarcocystis tenella* within goat heart cell. A = amylopectin granule; CW = cyst wall; GS = ground substance; MN = micronemes; N = nucleus; P = protrusion; R = rhoptry; HC = host cell
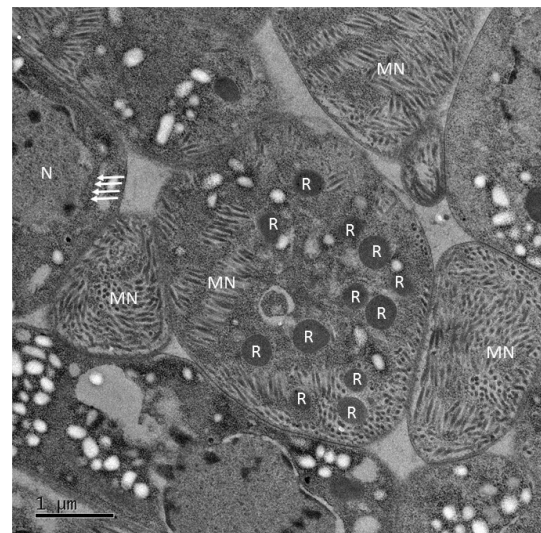

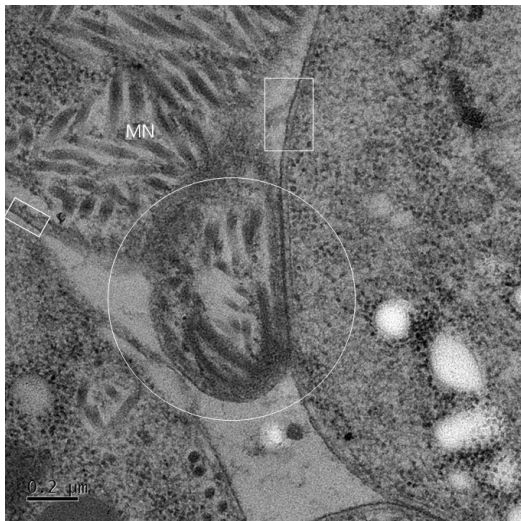Figure 3. Rophtries (R) and nuclear pores. Nuclear pores (arrows) are seen in cross-section. MN = micronemes

Figure 4. Parasites were surrounded by two membranes, an inner and outer one (squares). The conoid is marked by a circle. MN = micronemes

Figure 5. The membrane of the parasitic cyst has micro-pinocytic pits (arrows). Microfilaments are not found in the protrusion. P = protrusion

posterior part of the parasite contained the Golgi apparatus, the nucleus, the cisternae of granular endoplasmic reticulum, numerous amylopectin granules and plentiful free ribosomes (Figures 2 and 6). The cisternae of the granular endoplasmic reticulum were elongated (Figure 6). The nucleus had the usual appearance with a large nucleolus and the nuclear envelope had nuclear pores which were easily distinguishable (Figure 3). The presence of numerous amylopectin granules was characteristic of the posterior part of the bradyzoites (Figure 8). Sarcocyst morphology was compared with previous reports of sarcocysts found in sheep (Son et al. 2009). Based upon comparisons to previous reports
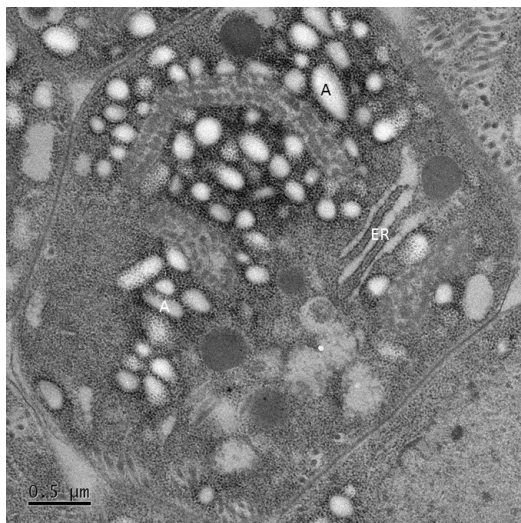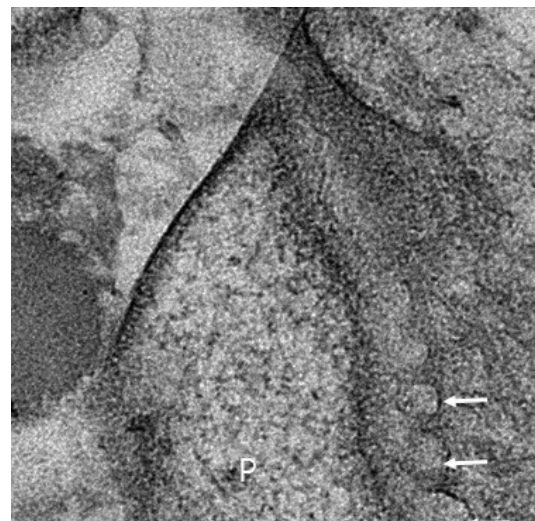
(Son et al. 2009), we identified the sarcocysts of Korean native goat as those of *S. tenella*.

Evaluation of PCR products electrophoresed on 1% agarose gesl showed a single band of around 1400 bp without any non-specific bands (Figure 9). The ITS2 region (1448 bp) of 18S rRNA in *Sarcocystis* specimens from Korean goats were identified and compared with *S. tenella*, and *S. capracanis*, respectively (Table 1). Partial ITS2 sequences of the *Sarcocystis* specimens contained sequences which placed them in the same clade as *S. tenella*. The sequence of *Sarcocystis* specimens was shown to vary from *S. capracanis* at 12 sites, and from *S. tenella* at two sites (Table 1). The variable sites

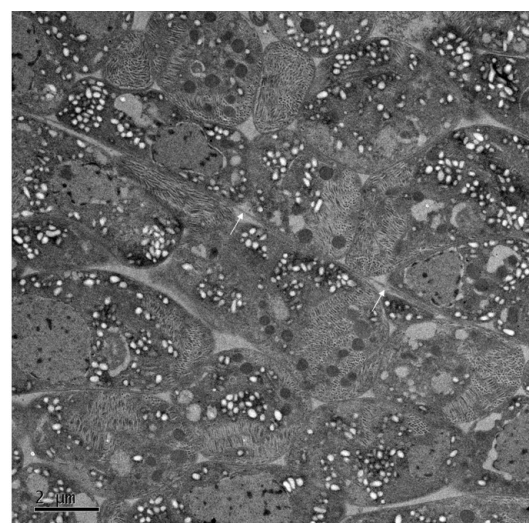Figure 6. The two cisternae of the ergastoplasm (ER) are seen. A = amylopectin

Figure 7. Inner part of a parasitic cyst of *S. tenella*. The inner part of a cyst is separated by septa (arrows)
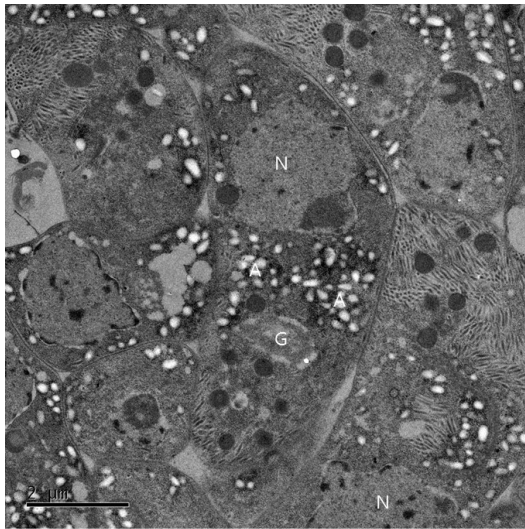
Figure 8. In the posterior part, the Golgi apparatus (G) and the nucleus (N) are evident

did not match with *S. capracanis* or *S. tenella.* The identification was also supported by the fact that they belonged to the same clades as *Sarcocystis* in the phylogenic tree (Neighbour-Joining method) based on the ITS2 rRNA gene. When the sequences of *Sarcocystis* specimens were compared with genes from *Sarcocystis* species (Figure 10), the sequences of *Sarcocystis* specimens were identified as a species that genetically matched *S. tenella* (KC029737.1, KC029734.1, KC029735.1), and was close to *S. capracanis* (L76472.1) and *S. alces* (KF831273.1, KF831274.1).

DISCUSSION

Sarcocystis species have been detected in muscle organs including heart, oesophagus, diaphragm, and skeletal muscles in intermediate hosts (Buxton
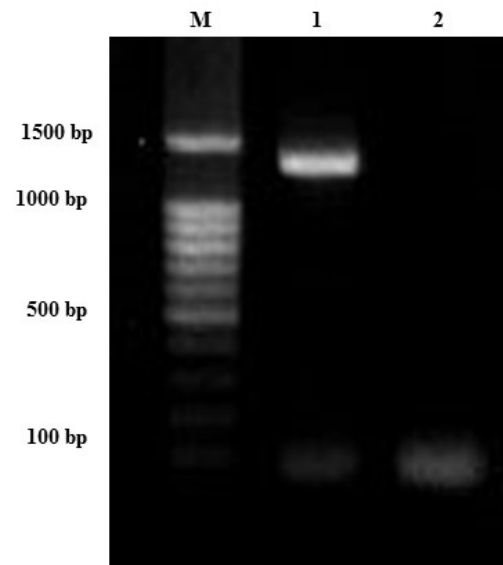

Figure 9. Agarose gel electrophoresis analysis of PCR products of from the *Sarcocystis* specimen. M = 100 bp DNA ladder; Lane 1 = 18S rRNA gene of *Sarcocystis* specimen; Lane 2 = negative control (distilled water)

1998; Daryani et al. 2006), and the most common tissue is the tongue muscle of intermediate hosts (Schmidtova and Breza 1992; Fukuyo et al. 2002; Gokpinar et al. 2014). Moreover, it was reported that muscle showed a 94.7% rate of infection in cattle (326 of 344), which included 93.3% infection rate (321 of 344) in heart, 87.5% (301 of 344) in diaphragm, 83.7% (288 of 344) in tongue, 85.1% (293 of 344) in oesophagus, 80.2% (276 of 344) in abdomen and 87.2% (300 of 344) in skeletal muscle (Hamidinejat et al. 2010). When the sarcocysts can be detected in the tissue, microscopic cysts of *Sarcocystis* spp. can be confused with tissue cysts of *Toxoplasma gondii* (Dubey et al. 1989). Beyond microscopic analysis, immunohistochemical and other methods are sometimes necessary to distin-
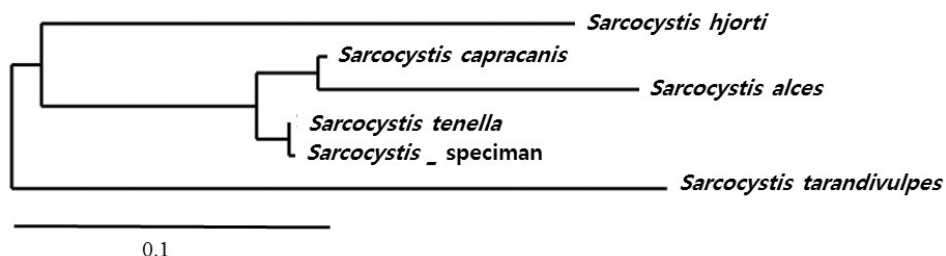
Figure 10. Phylogenetic analysis of the 18S rRNA gene sequences from *Sarcocystis* species and their evolutionary relationships to 36 taxa (1.5 kb). The sequences were analysed using DNASTAR, Clustal W and MEGA 6. Using the neighbour-joining methods a pairwise guide tree was created with multiple alignment parameters and bootstrap values with 1000 replications. The arrow indicates that the infected samples collected from cardiac muscles of Korean native goat, *Capra hircus coreanae*, contain *S. tenella*

Table 1. Multiple sequence alignment from *Sarcocystis* spp.

S. specimen	------------------ACCGTGGTAATTCTATGGCTAATACATGCGCAAATATCCTTTGTGTGAAAAAGGTGATTCATAGTAACCGAACGGATCGCATTATGGTCATTCTTTTGTAAT
S. tenella	------------------ACCGTGGTAATTCTATGGCTAATACATGCGCAAATATCCTTTGTGTGAAAAAGGTGATTCATAGTAACCGAACGGATCGCATTATGGTCATTCTTTTGTAAT
S. capracanis	------------------ACCGTGGTAATTCTATGGCTAATACATGCGCAAATATCCTTTGTGTGAAAAAGGTGATTCATAGTAACCGAACGGATCGCATTATGGTCATT----TTCAAT
	**** **
S. specimen	GGCTGGCGATAGATCATTCAAGTTTCTGACCTATCAGCTTTCGACGGTAGTGTATTGGACTACCGTGGCAGTGACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGA
S. tenella	GGCTGGCGATAGATCATTCAAGTTTCTGACCTATCAGCTTTCGACGGTAGTGTATTGGACTACCGTGGCAGTGACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGA
S. capracanis	GGCTGGCGATAGATCATTCAAGTTTCTGACCTATCAGCTTTCGACGGTAGTGTATTGGACTACCGTGGCAGTGACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGA
S. specimen	AACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACTCAGGGAGGTAGTGACAAGAAATAACAACACTGGAAATTTTATTTCTAGTGATTGGAATGATGGGA
S. tenella	AACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACTCAGGGAGGTAGTGACAAGAAATAACAACACTGGAAATTTTATTTCTAGTGATTGGAATGATGGGA
S. capracanis	AACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACTCAGGGAGGTAGTGACAAGAAATAACAACACTGGAAATTTTATTTCTAGTGATTGGAATGATGGGA
S. specimen	TTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATGTCTGCTGGAAGCAATCAGTCCGCCCTATTTGTAGGGTGTGCACTTGATGAATTCTGGCATTT
S. tenella	TTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATGTCTGCTGGAAGCAATCAGTCCGCCCTATTTGTAGGGTGTGCACTTGATGAATTCTGGCATTT
S. capracanis	TTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATGTCTGCTGGAAGCAATCAGTCCGCCCTATTTGTAGGGTGTGCACTTGATGAATTCTGGCATTT
S. specimen	TTGTCCCAATGATGTAACGGGGCGGTGGTACTCGGTGAAAGCCGAGTATCCTGTTTTCCGATTATTGGGATACTGTTACTTTGAGAAAATTAGAGTGTTTGAAGCAGGCTAATTGCCTTG
S. tenella	TTGTCCCAATGATGTAACGGGGCGGTGGTACTCGGTGAAAGCCGAGTATCCTGTTTTCCGATTATTGGGATACTGTTACTTTGAGAAAATTAGAGTGTTTGAAGCAGGCTAATTGCCTTG
S. capracanis	TTGTCCCAATGATGTAGCGGGGCGGTGGTATTCGGTGAAAGCCGAGTATCCTG--TTCCGATTATTGGGATACTGTTACTTTGAGAAAATTAGAGTGTTTGAAGCAGGCTAATTGCCTTG
	* * **
S. specimen	AATACTGCAGCATGGAATAACAATATAGGATTTCGGTTCTATTTTGTTGGTTTCTAGGACTGAAATAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAAT
S. tenella	AATACTGCAGCATGGAATAACAATATAGGATTTCGGTTCTATTTTGTTGGTTTCTAGGACTGAAATAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAAT
S. capracanis	AATACTGCAGCATGGAATAACAATATAGGATTTCGGTTCTATTTTGTTGGTTTCTAGGACTGAAATAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAAT
S. specimen	TCTTAGATTTGTTAAAGACGAACTACTGCGAAAGCATTTGTCAAATATGTTTTCATTAATCAAGAACGAAAGTTAGGGGCTCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAAC
S. tenella	TCTTAGATTTGTTAAAGACGAACTACTGCGAAAGCATTTGCCAAAGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGCTCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAAC
S. capracanis	TCTTAGATTTGTTAAAGACGAACTACTGCGAAAGCATTTGCCAAAGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGCTCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAAC
	* *
S. specimen	TATGCCGACTAGAGATAGGAAAATGTCATTTTGCTGACTTCTCCTGCACCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGAC
S. tenella	TATGCCGACTAGAGATAGGAAAATGTCATTTTGCTGACTTCTCCTGCACCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGAC
S. capracanis	TATGCCGACTAGAGATAGGAAAATGTCATTTTGCTGACTTCTCCTGCACCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGAC
S. specimen	GGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATGGGAAGGATTGACAGATTGATAGCTCTTTCTTGATTCTATGGGTGG
S. tenella	GGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATGGGAAGGATTGACAGATTGATAGCTCTTTCTTGATTCTATGGGTGG
S. capracanis	GGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATGGGAAGGATTGACAGATTGATAGCTCTTTCTTGATTCTATGGGTGG
S. specimen	TGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCTTAACCTGCTAAATAGGATCAGAAACACTTTATTGTGTTTTTGTATCACTTCTTA
S. tenella	TGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCTTAACCTGCTAAATAGGATCAGAAACACTTTATTGTGTTTTTGTATCACTTCTTA
S. capracanis	TGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCTTAACCTGCTAAATAGGATCAGAAACACTTTATTGTGTTTTTGTATCACTTCTTA
S. specimen	GAGGGACTTTGCGTGTCTAACGCAAGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCTGCACGCGCGCTACACTGATGCATCCAACAAGTTGTTGAAA-----
S. tenella	GAGGGACTTTGCGTGTCTAACGCAAGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCTGCACGCGCGCTACACTGATGCATCCAACAAGTTGTTGAAA-----
S. capracanis	GAGGGACTTTGCGTGTCTAACGCAAGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCTGCACGCGCGCTACACTGATGCATCCAACAAGTTGTTGAAA-----

guish parasite species. Using heart tissue of the slaughtered goats, we attempted to determine the identity of the parasite. The source of infection was identified as *S. tenella* by morphological and molecular analysis in all positive samples. The sarcocyst infection rate was at about 3%. Interestingly, there have not been any reports of natural infection by sarcocysts in Korean native goats until now.

The size and shape of sarcocysts is determined by the class and age of the *Sarcocystis* species. *S. cruzi* is always of microscopic size, but *S. gigantea* or *S. muris* have been detected macroscopically (Dubey et al. 1989). In general, the shape of sarcocysts can be filamentous, elongated or globular, and the shape and size of cysts is also dependent upon the shape of host cells. Macroscopic sarcocysts are nearly always found in skeletal muscle or oesophageal muscle (Dubey et al. 1989). Regarding the ultrastructure of *S. tenella,* most

findings from our study matched previously published works (Dubey et al. 1989; Dubey and Odening 2001). Using TEM analysis, the ultrastructure of the sarcocyst wall was classified into 24 specific types based upon a system introduced by Dubey et al. (1989). Also, 13 additional types were subsequently described by Dubey and Odening (2001). Based on the size, shape and spacing of cyst wall villi with or without microtubules within the villi, sarcocyst species have been classified with respect to their ultrastructural morphology. The sarcocyst wall, ground substance underlying it, and the septae are remnants of the merozoite. The metrocytes and bradyzoites within the sarcocyst are formed from endodyogenic divisions of the merozoite nucleus. Other than the sarcocyst wall, ultrastructural morphology is fairly consistent among species; a few species lack septae and the density of bradyzoites within the sarcocysts may

vary between species (Dubey and Odening 2001). Mature sarcocysts may contain both metrocytes and bradyzoites, although bradyzoites predominate (Dubey et al. 1989). Although light microscopic examination is commonly used for identification of sarcocysts, electron microscopy has been used to identify *Sarcocystis* spp. according to their wall structure (Haziroglu et al. 2002). There has also been a report that cyst wall structure is important for the identification of *Sarcocystis* species (Heckeroth and Tenter 2007). For example, *S. arieticanis* have hair-like projections in the walls of their cysts that are 5–9 μm long (Dubey et al. 1989; Heckeroth and Tenter 2007). In Egyptian goats, the primary cyst wall of *S. capracanis* has non-branched finger-like protrusions including internal fibrillar elements, and its protrusions were shown to be irregular (Morsy et al. 2011). Unlike *S. arieticanis* or *S. capracanis*, *S. tenella* has 3.5 μm-long villous protrusions in the walls of their cysts, and its protrusions were shown to be round, quadrate or flattened without microtubules. Interestingly, the villous protrusions of *S. tenella* reveal different patterns from *S. capracanis.* The ground substance of *S. specimens* is located at the base of the protrusions, and is a common characteristic for all described *Sarcocystis* species. It extends inside the cyst cavity in the form of large numbers of septa which divide the cavity into numerous compartments containing the cyst merozoites. This is a general characteristic of all described *Sarcocystis* species (Abdel-Ghaffar et al. 1994; Mehlhorn 2001). However, the ground substance is not found in cysts of other species such as *T. gondii* (Scholtyseck et al. 1974). Numbers of rhoptries in each bradyzoite may differ among *Sarcocystis* species (Dubey et al. 1989). Although bradyzoites and metrocytes contain the same typical cell organelles such as nucleus, endoplasmic reticulum, mitochondria and inclusion bodies, metrocytes lack the distinctive apical complex, micronemes, and rhoptries found in bradyzoites (Dubey et al. 1989). In our study, the apical complex, numerous micronemes and up to 12 rhoptries were located in the anterior part of bradyzoites.

The 18S rRNA gene has been used to study phylogenetic relationships because ribosomal DNA sequences exhibit high repetition as well as variable regions flanked by more conserved regions (Hillis and Dixon 1991). These characteristics have been exploited for diagnostic purposes (Leon-Regagnon et al. 1999). Phylogenetic analyses of ribosomal RNA sequences have provided support for paraphyly of *Sarcocystis* beyond the morphological identification (Ellis et al. 1995). Also, ITS-2 sequences have been used to identify *S. tenella* (Da Silva et al. 2009). Following the above methods, we confirmed that the *Sarcocystis* specimens from Korean goats are *S. tenella*, and we could distinguish them from other *Sarcocystis* species such as *S. capracanis*, *S. alces*, *S. hjorti*, and *S. tarandivulpes.*

Although sheep have been described to be intermediate hosts of *S. tenella*, there is no report of domestic goats acting as intermediate hosts of this parasite (Collins et al. 1979; O'Donoghue and Ford 1986; O'Donoghue 1996). To date, three species of *Sarcocystis* (*S. capracanis*, *S. hircicanis* and *S. caprafelis*) have been detected in domestic goats. The present finding was unexpected and broadens our knowledge of *S. tenella* epizootiology by demonstrating that the domestic goat may also be an intermediate host of this parasite*.

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