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Case Report

First Molecular Identification of *Sarcocystis ovicanis* (Protozoa, Apicomplexa) in the Brain of Sheep in Iran

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Abstract

Background: The objective of the present study was to survey the presence of *Sarcocystis* in sheep's brain in North Khorasan Province.

Methods: In general, 80 samples of sheep's brain were collected from slaughtered sheep in slaughterhouses of North Khorasan Province. Tissue digestion method was used for observing bradyzoites in tissues. Histopathological processing tracing *Sarcocystis* and ensuing structural change in the brain tissue were conducted. PCR analysis was conducted on all the brain samples. Sequencing was done for one PCR product. Genotype was identified by Blast search and homology analysis.

Result: *Sarcocystis* spp. was found in one of the brain samples (1.25%) using tissue digestion method. The presence of bradyzoite was also confirmed in the prepared histopathological sections. PCR analysis was positive in one of samples. Genotyping of one sample proved that *Sarcocystis* species was *Sarcocystis ovicanis* and the nucleotide sequence of this parasite was deposited in the GenBank database under accession number No.KF489431.

Conclusion: *Sarcocystis ovicanis* can involve brain tissue of sheep and consequently causes clinical symptoms.

Introduction

Sarcocystis spp. are intracellular protozoan parasites infecting a wide range of animals, even humans. More than 100 species of *Sarcocystis* can infect wildlife and

livestock animals (1). There are two definitive and intermediate hosts with two sexual and asexual stages in the life cycle of this parasite. The sexual stage is performed in the epithelial

cells of the definitive host and a sexual stage in the tissues of intermediate host (2). *Sarcocystis* is pathogenic for livestock such as sheep and cattle and causes enormous economic losses (3,4).

Sheep are intermediate host of some species of *Sarcocystis*: *S. ovicanis*, *S. gigantea* (5). *S. ovicanis* and *S. gigantea* have worldwide spread (5-8). *S. ovicanis* is transmitted by dog and is pathogenic (5).

S. ovicanis can create economic problems due to reduced meat, milk or wool (5, 9).

The prevalence of *Sarcocystis* infection in slaughtered cattle and sheep were between 70-100% (10-14). In Iran, 100% of animals were infected with *Sarcocystis* (3,4,6). Different species of *Sarcocystis* have been isolated from animals in the world (15-18). Dalimi found *S. gigantea* and *S. articanis* in sheep using the PCR-RFLP method in Qazvin province (7). Kia et al. reported *S. miescheriana* from boar (2). In East Azerbaijan Province, *S. ovicanis* was reported from sheep (8).

Since there was no information regarding *Sarcocystis* infection in sheep's brain in Iran, the main objective of the present research was to investigate the presence of *Sarcocystis* in sheep's brain.

Materials and Methods

Sampling method

This descriptive cross-sectional study was conducted from 2012 to 2013. Overall, 80 brain samples were collected from 80 slaughtered sheep in slaughterhouses of North Khorasan Province, Iran.

Digestion method

Tissue digestion method was used for observing bradyzoites in the brain samples. Seventy grams of each tissue were ground and digested in 1.5% HCL acid and 0.5% pepsin at 29 °C overnight. The digested samples were filtered through mesh and centrifuged 1500 RPM for 10 min. Then, the supernatant fluid

was discarded and sediment was stained with Gimsa and examined microscopically for detecting bradyzoites of *Sarcocystis*.

Histopathological method

Histopathological method was undertaken for observation of bradyzoites in brain tissue samples. The samples were fixed in 10% formalin, the paraffin block was prepared and 4 to 5 μ sections were provided, and then stained with Haematoxylin and Eosin method. The slides were examined for detecting bradyzoites under light microscope at 100X magnification. Photographs were taken with an Olympus digital camera.

DNA extraction and PCR amplification

For DNA extraction, a small piece of samples was selected and then their DNAs were extracted through tissue DNA extraction kit (Cinnagen Company). PCR Analysis was performed on all tissue samples using Sar primers of 18srRNA gene including Sar-F1 Forward GCAC TTGATGAAT TCTGGCA and Sar-R1 Reverse CACCACCCATAGAATCAAG as described previously (7).

PCR Reaction was carried out in 30 ml of Ampliqone (TaqDNA polymers Mastermix RED, Denmark). Twenty-five microliters of Taq Master mix were used with 10ng template DNA, 0.1 μ M of each primer and distilled water. Cycles of PCR were set up as follows: Pre-denaturation step at 94 °C for 5 min and 30 cycles of denaturation at 94°C for 45 Sec, annealing at 55 °C for 1 min and extension at 72 °C for 1 min with an elongation step of 7 min at 72 °C at the last cycle.

PCR product electrophoresis was done on 2% agarose gel stained with ethidium bromide (0.5 μ g/ml) and visualized under the UV light.

Sequencing and Genotyping of isolates

The PCR product was purified using Column-based purification kit and then sequenced through automatic sequencer of the Korean Macrogen Company. The obtained sequence was edited using the Bioedit software

program. Genotype identification was done by comparing with available *Sarcocystis* DNA sequences in the GenBank based on sequence analysis of 18s rRNA region.

Results

Results obtained from tissue digestion method indicated that one of brain samples (1.25%) was infected with bradyzoites of *Sarcocystis* (Fig.1).

Histopathological analysis revealed that there were a few dispersed bradyzoites of *S. ovis* in different regions of the brain both in white and gray matter (Fig. 2, 3). These bradyzoites had elliptical shape and basophilic properties in H&E staining (Fig. 4, 5). There were a few pyknoticnucleous of neuroglia but, we saw no evidence of hemorrhage, perivascular lymphocytic cuffing, myelin sheath invasion by macrophage and encephalitis in histopathological analysis. Examinations of some regions of the brain (e.g. cerebellum) from histopathological view showed no structural changes and were completely free from bradyzoites.

PCR analysis was performed on all brain samples. The PCR reaction, using a pair of primer, yielded an expected band on agarose gel. 600 bp band was only observed in one of brain samples (Fig. 6).The obtained DNA sequence was analyzed using Blast software and the isolate was identified as *S. ovis*. The nucleotide sequence of this parasite was deposited in the GenBank database under accession No.KF489431.

Discussion

Sarcocystis is pathogenic for livestock such as sheep and cattle. Previous studies in Iran and the world have indicated that these animals can be infected with *Sarcocystis* (6, 19, 10-14, 18, 20-22). Other studies in different provinces of Iran have found that 85%-100% of cattle and sheep had *Sarcocystis* (3, 4, 6). In

Iran, samples were collected from other organs of sheep such as muscle, tongue, heart and diaphragm and *Sarcocystis* was seen in all organs, but, in this study, *Sarcocystis* was isolated from sheep's brain tissue for the first time (3,4,6-8). Also, no lesions were observed on gross examination section of cerebrum, cerebellum, mid brain and medulla. Myeloencephalitis caused by *S. ovis* is associated with predominantly neurologic signs, particularly, paresis or paralysis of pelvic limbs, trembling, incoordination or excitement (23). Ovine *Sarcocystis* spp. can create fever, anorexia, anemia, myositis and encephalitis (24). Severity of clinical signs produced by *S. ovis* depends on the dose of ingested sporocyst and the immune system of host (25-28). *S. ovis* can lead to acute sarcocystosis in uninfected sheep (29).

Nonspecific infection symptoms include fever, anorexia, tachycardia and anemia. The condition progresses rapidly in fatal cases and death generally occurs within 7 days of the onset of clinical signs (30). We found anorexia in the infected sheep with *Sarcocystis*.

Different studies were done worldwide regarding pathogenicity of *Sarcocystis* in the brain tissue (23, 24, 30, 31). *Sarcocystis*-like protozoan can cause ovine myeloencephalitis (23). *S. ovis* can cause ovine myeloencephalitis (32). Henderson et al. identified *Sarcocystis* spp. in brain tissue by histopathological method (31).Morgan isolated *Sarcocystis* spp. from sheep's brain tissue (33). Different histopathological changes caused by *S. ovis* were identified in the brain tissue, such as perivascular lymphocytic cuffing, vascular degeneration, and myelin sheath invasion by macrophage (23, 24, 30, 31). But, in this study, the minimum histopathological changes were seen in the brain tissue samples including nucleospikyknosis of glial and bradyzoites. It appears the reason for the difference between histopathological data in different studies related to the number of eaten sporocyst by the animal.

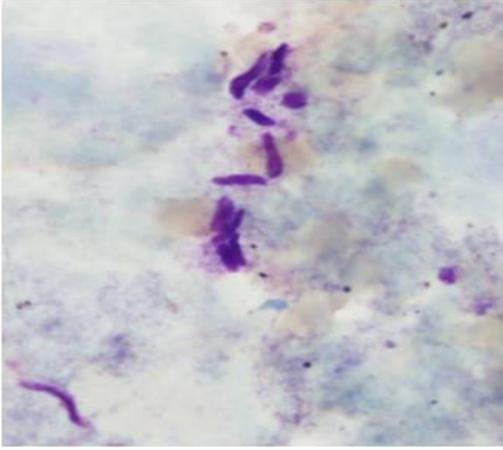


Fig. 1: Bradyzoite of *Sarcocystis* in sheep's brain prepared by digestion method.×100

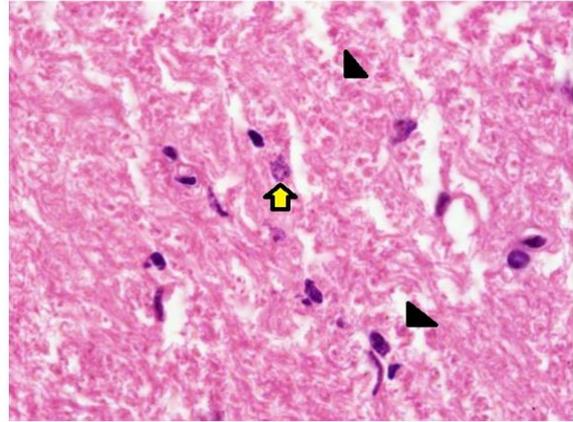


Fig. 2: White matter of brain tissue sample(cross section of myelinated axons indicated by arrowheads) infected by bradyzoites of *Sarcocystis ovicanis* and some glial cell pyknotic nucleus(arrow). H&E,×100

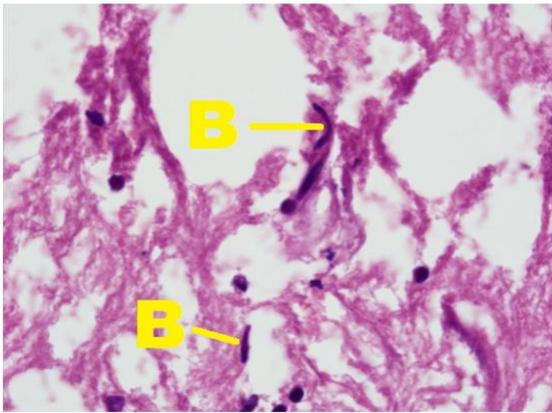


Fig. 3: Brain tissue samples with some elliptical and basophilic bradyzoites of *Sarcocystis ovicanis*(B). H&E,×100

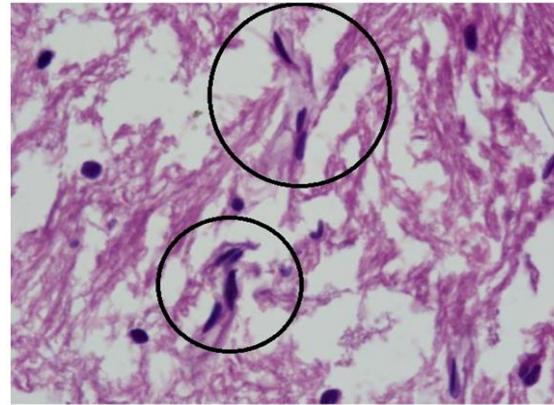


Fig. 4: Brain tissue samples with some elliptical and basophilic bradyzoites of *Sarcocystis ovicanis*. H&E,×100

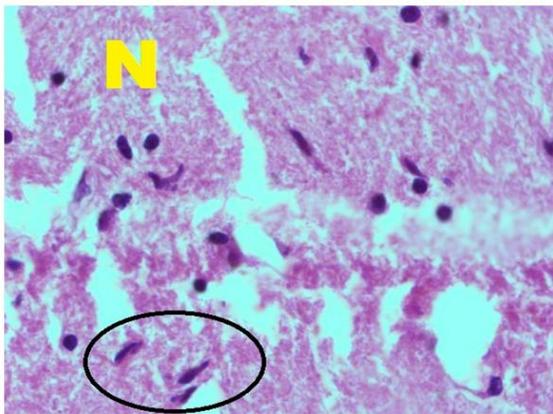


Fig. 5: Brain tissue samples with some elliptical and basophilic bradyzoites of *S. ovicanis* and surrounding neurophilic(N). H&E,×100

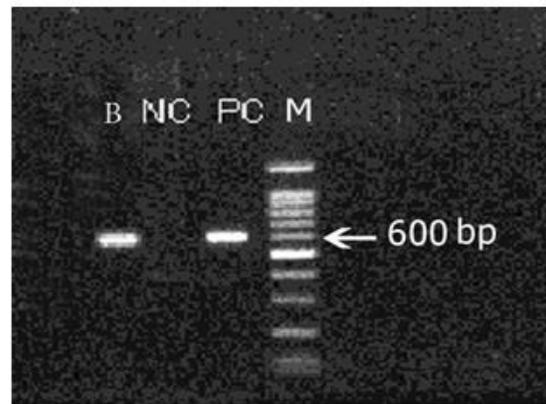


Fig. 6: PCR Product from extracted DNA of *Sarcocystis* sheep's brain, M=Molecular weight marker (100bp), PC=Positive Control, NC=Negative Control, B=Brain

Therefore the chance of distribution and entrance of bradyzoites into the brain tissue and its surrounding meninges are variable. *S. ovis* could cause encephalomyelitis in sheep and finally cause recumbence (34). The results of histopathological sections were confirmed by PCR analysis and sequencing. The finding showed that pathogenic species was *S. ovis*. *S. ovis* is thought to be the most pathogenic of the *Sarcocystis* species in sheep. We saw bradyzoites of *Sarcocystis* in brain tissue by digestion method and for the further confirmation histopathology method and PCR analysis were utilized. *S. ovis* was identified by molecular analysis and sequencing.

Conclusion

This study is the first report of *S. ovis* in sheep brain tissue and, it seems, we could identify the bradyzoites in sheep brain tissue by digestion and histopathological methods, but, it is necessary to use PCR analysis for final confirmation.

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