Veterinary Parasitology: Regional Studies and Reports 13 (2018) 193–197

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Short communication

Genetic variability within isolates of *Sarcocystis species* infecting sheep from Egypt

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ARTICLE INFO

Keywords:
Sarcocystis tenella
Sarcocystis gigantea
Sheep
Egypt
18S rRNA
Genetic variability

ABSTRACT

In order to explain the exact role of sarcocystosis, principally *Sarcocystis tenella*, in losses of the Egyptian sheep industry, a precise confirmation about the existence of different *Sarcocystis species* infecting that economically important animal is needed. Therefore, this work aimed to molecularly identify, as well as illustrate the genetic variability within isolates of *Sarcocystis* spp. infecting sheep from Egypt. Tissue specimens were collected from sheep slaughtered at 3 Egyptian provinces; Cairo, Dakahlia and Damietta. DNA was isolated from the harvested bradyzoites after peptic digestion for the positive sarcocysts infected specimens, and then PCR amplification using the 18S rRNA gene was carried out. PCR products were subjected to gel electrophoresis. DNA from 600 bp gel bands was purified and sequenced. The revealed sequences were compared to their similarities on Genbank, and analyzed both clusterally and phylogenetically. Two *Sarcocystis* spp. were identified, the macroscopic cyst forming *S. gigantea* and the microscopic cyst forming *S. tenella*. Nine *S. tenella* sequences were analyzed, resulting in 3 polymorphic sites as well as 3 different haplotypes. Clustering of the nine obtained *S. tenella* sequences in addition to another 23 *S. tenella* sequences on Genbank revealed low nucleotide (0.001780) diversity as well as negative value of the Taijma neutral index which are indicators for population expansion. Alignment and Phylogeny results illustrated very close relationship between *S. gigantea* and *S. moulei*, a goat specific species which rarely reported in sheep, and in turn proposed the cross transmission of the later species between sheep and goats.

1. Introduction

Sarcocystis spp. are prevalent apicomplexan protozoans with obligatory intracellular nature. Variable hosts could be infected, either as definitive or intermediate, depending on the prey-predator relationship. Sexually reproduced enteric stages were found in the gut of definitive hosts (dogs, cats and man), while a wide range of intermediate hosts, including farm animals, harbor sarcocysts which represent the tissue stages (Fayer, 2004). Sheep is reared worldwide. According to records of the ministry of agriculture and land reclamation, 5 463 169 sheep heads are reared in Egypt to be used for meat, milk and wool production. They are reared in an open system with frequent movement to agricultural lands for feeding on agricultural residues after harvest.

Multiple *Sarcocystis* spp. infection is common in many animals. Four species of *Sarcocystis* are known to infect sheep including the macroscopic cyst forming *S. gigantea* and *S. medusiformis*, and the microscopic cyst forming *S. tenella* and *S. arieticanis* (Dubey et al., 2015). The later two species transmitted through dogs and exhibited more pathogenic effect than the former species which transmitted via cats (Heckeroth and Tenter, 1998). Rare cases of *S. mihoensis* and *S. microps* were reported from sheep in Japan and China, respectively (Hu et al., 2017). Overall, sarcocystosis has a negative economic impact on the animal industry, through decreasing meat quality with subsequent downgrading or even condemnation of carcasses. In addition, the two pathogenic species are incriminated in variable cases of abortion, poor growth and acute fatal illness resulting in losses of several million dollars every year (Dubey, 1976).

Prevalence of sheep sarcocystosis varies from 0 to 100%, worldwide (Dubey et al., 2015). Whereas, high rates of infection (40–100%) were recorded in different Egyptian provinces (Ali, 1985; Elsayed, 1985; El-Ganiny, 1989; El-Saieh, 1998; Hassanien, 1992).

Based on the morphological characters using the light microscopy, Mahran (2009) reported the infection of sheep from Egypt with both of *S. gigantea* and *S. tenella.* Morphological characterization of *Sarcocystis* spp. mainly depends on using the transmission electron microscopy exhibiting the cyst wall type (Dubey et al., 2015). Although these procedures are beneficent, they are not exactly reliable in discrimination of closely related species (Hamidinejat et al., 2014). Recently,

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https://doi.org/10.1016/j.vprsr.2018.07.002
Received 9 December 2017; Received in revised form 25 May 2018; Accepted 3 July 2018
Available online 07 July 2018

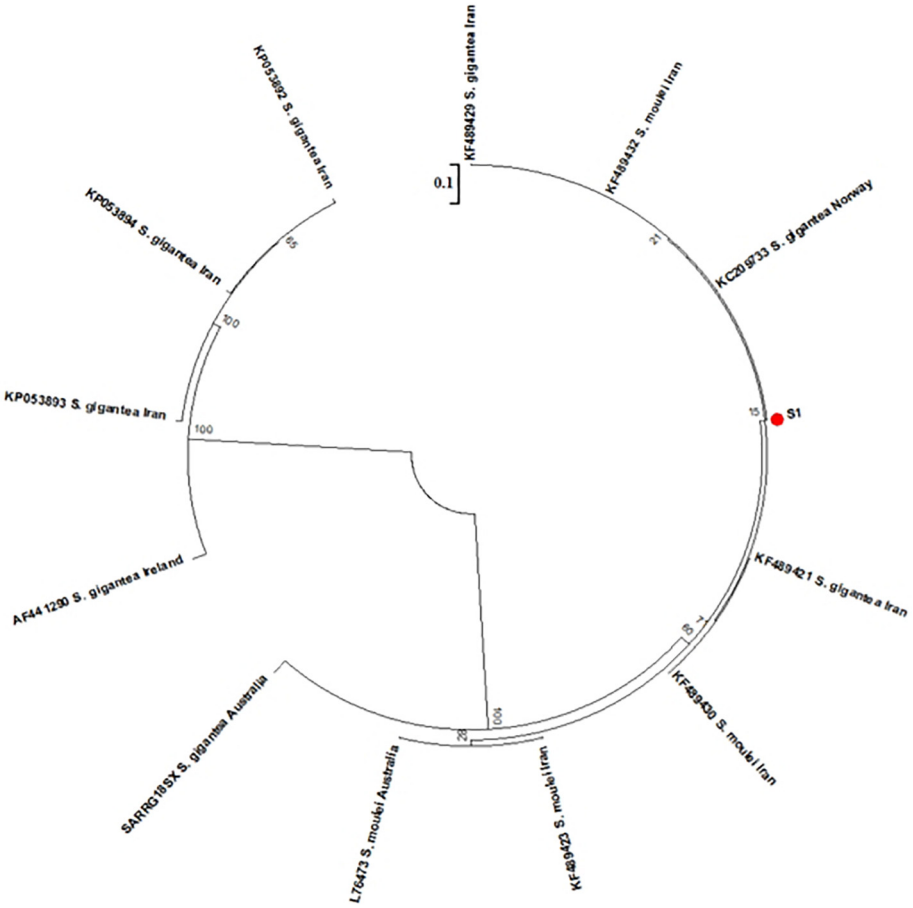

Fig. 1. Frequencies of 18S rRNA sequence sections of *S. gigantea* isolates of sheep from variable geographical regions including Egypt (S1). Six haplotypes were noted. The scale bar refers to evolutionary distances in substitutions per site.

accurate discrimination of different *Sarcocystis* spp. is relying on molecular techniques such as PCR-RFLP using variable genetic markers particularly 18S rRNA which is highly conserved gene (Hamidinejat et al., 2014). Hence, molecular characterization of different *Sarcocystis* spp. infecting sheep from Egypt is important.

Therefore, the present study aimed to identify the species and to illustrate the genetic variability, based on partial 18S rRNA gene sequences, within different isolates of sarcocysts from tissues of sheep reared in Egypt.

2. Material and methods

2.1. Samples and study area

Tissue specimens (esophagus, diaphragm and abdominal muscles) from 540 slaughtered sheep were collected during routine meat inspection in 3 municipal abattoirs, of high sheep slaughter density, at Cairo (Capital of Egypt) and two provinces in the Delta region (Dakahlia and Damietta). Samples were inspected for macroscopic sarcocysts by naked eyes, while the microscopic sarcocysts were visualized under a dissecting microscope using muscle compression technique (Gut, 1982). Fast 5 min staining of the fresh specimens with 10% Geimsa solution was perfect for better observation of the microscopic sarcocysts. Peptic digestion was conducted for the positive samples according to (Dubey et al., 2015). The harvested bradyzoites were washed 3 times in PBS and preserved at −20 °C until used.

2.2. DNA extraction and molecular analysis

The harvested bradyzoites were subjected to DNA isolation using QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Amplification of the 18S rRNA gene was achieved by using the primer pair Sar-F1 (5'GCACTTGATGAATTCTGGCA3') as forward and Sar-R1 (5'CACCACCCATAGAATCAAG3') as reverse (Bahari et al., 2014). The reaction was carried out in a total volume of 25 µl containing 1 µl (20 pmol) of each primer, 12.5 µl Emerald Amp GT PCR master mix (Takara Bio Inc.), 6 µl template DNA and 4.5 µl nuclease free water. The profile of thermal cycles was as follows: 5 min at 94 °C as an initial hot start step, followed by 35 cycles of 45 s at 94 °C, 1 min. at 55 °C, 1 min. at 72 °C, and final extension for 10 min at 72 °C. Positive and negative controls were included in each experiment.

PCR products were visualized on 1.5% agarose gel stained with ethidium bromide. Gel Bands of about 600 bp were cut off the gel. Purified DNA was extracted using QIA quick PCR product extraction kit. (Qiagen Inc. Valencia CA), and then sequenced in an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA).

2.3. Data analysis

Sequences were aligned and truncated slightly at both ends using Bioedit software; therefore the majority of sequences started and ended at the same homologous nucleotide positions. The revealed sequences were subjected to BLAST (http://www.blast.ncbi.nlm.nih.gov). Different diversity indices (haplotype and nucleotide), Tajima D neutral index (Tajima, 1989), the UPGMA (clustering) tree and the Maximum Pairsmony phylogenetic tree were initiated using the software MEGA