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Survey of Sarcocystis Infection in Slaughtered Sheep in Isfahan, Qom and Shahre-Kord, Iran

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Abstract: Sarcocyst species are intracellular protozoan parasites with an intermediate-definitive host life cycle based on a prey-predator relationship. A sexual stages develop in intermediate hosts after they ingest the oocyst stage from definitive-host feces and terminate with the formation of intramuscular cysts (Sarcocystis). The present study was carried out to find out the incidence of sarcocystosis (in sheep) in three slaughterhouses in Iran. A total of 650 muscle samples of 325 sheep from 4 different age groups were surveyed by the digestion and impression smear examination methods for the presence of Sarcocystis. After slaughtering, esophagus and diaphragm were examined for macroscopic cyst of Sarcocystis. For microscopic cysts, the samples were taken from each one of these tissues for impression smear (Dob smear) and digestion. Macrocysts were found in 4.3% (14/325) of sheep and the highest prevalence was detected in the diaphragm in all age groups. Microcysts were present in 92% (299/325) of sheep. There was no significant difference ($p>0.05$) statistically between sensitivities of two diagnostic methods (digestion and impression smear) in detecting Sarcocystis species. These results reveal the fact that the environment is heavily contaminated with sporocysts and the ingestion of sporocysts by sheep begins from the young ages in Iran.

Key words: Sarcocystis, slaughterhouse, sheep, incidence, macroscopic cyst, Iran

INTRODUCTION

Sarcocystis species are obligatorily intracellular protozoa with a typical coccidian life cycle consisting of merogony, gamogony and sporogony. Sarcocysts in meat eaten by a definitive host initiate sexual stages in the intestine that terminate in oocysts excreted in the feces. The asexual stages including sarcocysts can stimulate a strong inflammatory response. Sheep may be infected by four species of Sarcocystis: *Sarcocystis ovicanis* (Synonymous = *S. tenella*) and *Sarcocystis arieticanis* are pathogenic, form microscopic cysts and are transmitted via canids, *Sarcocystis ovifelis* (Synonymous = *S. gigantea*) and *Sarcocystis medusiformis*, non-pathogenic species, form macroscopic cysts and are transmitted via felids (Dubey *et al*., 1989). The prevalence's of ovine Sarcocystis species detected in different provinces of Iran have also ranged from 55-100%.

Sarcocystosis has been reported in sheep from various countries such as Iran (Valinezhad *et al*., 2008), United States (Fayer, 2004), Sudan (Ginawi and Shommein, 1977) and Argentina (More *et al*., 2008).

The main objective of the study reported here was to determine the prevalence of ovine Sarcocystis species in Iran by using the digestion and impression smear examination methods. In addition, the influences of age and sex on the prevalence of Sarcocystis species were also investigated.

MATERIALS AND METHODS

Muscle samples were collected between August, 2008 and July, 2009 from 325 sheep both slaughtered at various abattoirs in Iran and necropsy in Share-kord Veterinary Faculty laboratory complex <6 months of age for the purpose of diagnosis. About 3 slaughterhouses are in the center of 3 provinces of center of Iran. All sheep had been raised in different locations of Iran. A total of 650 muscle samples from the esophagus and diaphragm in some of them were examined for the presence of Sarcocystis species by the digestion and direct smear techniques. Sheep's age were estimated by looking at its teeth. Sheep were surveyed in 4 groups according to their ages: up to 12 months, >1-2, >2-3 and >3 years. They were initially examined by the naked eye for the presence

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of macrocysts. The found cysts were counted and measured for their dimensions. Later, the samples were divided into two sets: one set for digestion procedures, the other set for impression smear examinations. For the digestion, 10 g of each muscle sample were homogenized in a blender and digested in a NaCl-buffered 6% pepsin solution (Germany; Merck) as described by Erber. After 10-15 min incubation (Memmert, Modell 100-800), the digest was sieved through a nylon-meshed tea strainer and centrifuged within the tubes with conic bottom at 1500 rpm for 10 min (Hettich EBA 20). After discarding the supernatant fluid, 1-2 drops from the sediment at the bottom were examined microscopically for Sarcocystis microcysts.

For impression smear examinations, About 1 g of muscles was cut into small pieces, approximately 3-5 mm thick and crushed strongly between 2 glass slides and after staining with Gimsa (Germany; Merck) examined under the microscope (400x).

Both the sensitivities of two diagnostic methods (digestion and impression smear) and the differences in prevalences of microcysts according to the muscles of different organs and sex in sheep from four different age groups were evaluated statistically by using the z-test and ANOVA (Beyazit *et al*., 2007). $p<0.001$ were considered significant.

RESULTS AND DISCUSSION

Sarcocystis macrocysts were found in 4.3% (28/650) of 325 sheep. The overall prevalence of Sarcocystis microcysts in all sheep with or without macrocysts was detected as 92% (598/650) (Fig. 1). No significant difference ($p>0.05$) was statistically found between male and female in three slaughterhouses (Fig. 2). The highest prevalence of microcysts was detected in the diaphragm in three slaughterhouses (Fig. 3). Sarcocystis is worldwide in distribution. Parasites belonging to this genus have been reported from numerous mammals, especially sheep, cattle, buffaloes and pigs. The rate of infection reach to 100% in sheep and cattle in the United States (Fayer, 2004) and a high frequency of infection (91.6%) was also reported in goats in Sudan (Ginawi and Shommein, 1977) and Argentina (More *et al*., 2008). There are a few reports about prevalence of Sarcocystis in domestic animals in Iran, the rate of infection in sheep (6.67%) and goats (12.25%) in Khoram-Abad has been reported (Atashparvar *et al*., 2001) and prevalence rate of 3.58% in sheep and 0.13% in goats has been reported in Kerman (Radfar and Khosravi, 2001). The prevalence of *Sarcocystis* sp. was 83.6% by gross and histopathological examinations in camels slaughtered in the Mashhad Slaughterhouse, Eastern Iran (Valinezhad *et al*., 2008).

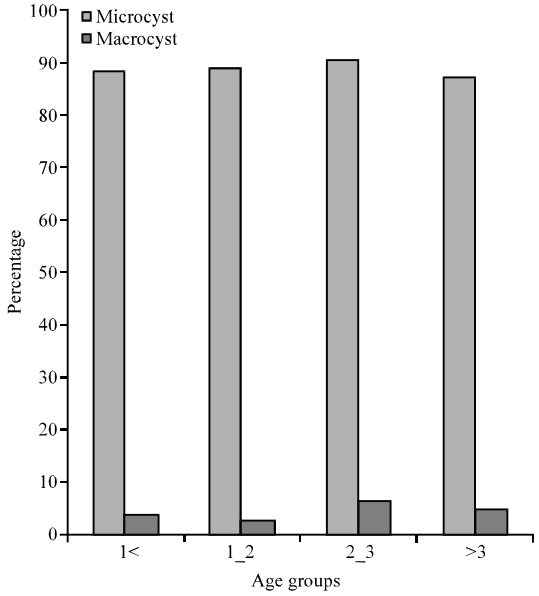

Fig. 1: Prevalence of sarcocyst macrocyst and microcyst from four different age groups in slaughtered sheep in Iran

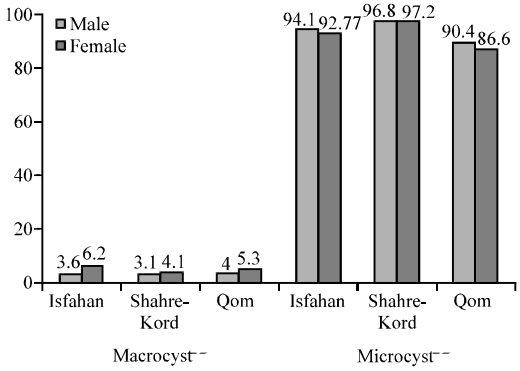

Fig. 2: Prevalence of sarcocyst macrocyst and microcyst according to the sex in three slaughterhouses, Iran

Also the rate of infection in goats 98.97% were diagnosed as positive for Sarcocystis species by the impression smear method and all 294 were positive by the digestion method. in Kerman, province has been reported (Valinezhad *et al*., 2008). In the present study, Sarcocystis macrocysts were found in 4.3% (28/650) of 325 sheep. The highest prevalence (16%) recorded in the diaphragm was similar to the observations of other some researchers, except for that of Al-Hoot *et al*. (2005), Britt and Baker (1990) and Latif *et al*. (1999) who detected the highest prevalence of cysts in the esophagus. Macrocysts have been reported to be found mostly in adult sheep (Abo-Shehada, 1996; Svobodova and Nevole, 1990). In this study, the prevalence of macrocysts increased with age (Fig. 1).

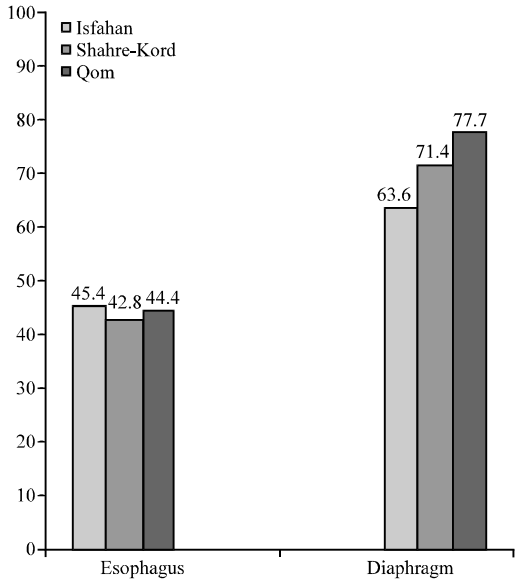

Fig. 3: Prevalence of Sarcocystis microcysts according to the tissue in three slaughterhouses in Iran

Macrocyst was found in 1 sheep under 1 year old, supporting the finding of Svobodova and Nevole (1990) who reported macrocysts in a young sheep. This finding suggests that macrocysts may develop in a time <1 year unlike the previous observation that they need at least 1 year to develop (Munday, 1978).

Microscopic ovine Sarcocystis species have been observed to be more prevalent than macroscopic cyst-forming species in Iran (Shekarforoush *et al*., 2005) and other many countries (Dubey *et al*., 1988; Martinez-Moreno *et al*., 1989). The differences in prevalence between the age groups were no significant ($p<0.05$) in sheep aged up to 1 year. Microcysts were found in 23 (46%) lambs in this age group. While the prevalences of microcysts in the esophagus and diaphragm was similar (Fig. 3). The prevalences of microcysts recorded in lambs in different countries have been reported as 22.6% in those fewer than 6 months of age in Austria, 58.33% in those fewer than 3 months of age and 79.05% in those between 3 and 12 months of age in Spain (Martinez-Moreno *et al*., 1989), 35.67% in Czechoslovakia (Svobodova and Nevole, 1990) and 18.3% in the United States (Seneviratne *et al*., 1975). In some studies (Valinezhad *et al*., 2008) performed in Iran, the prevalences of Sarcocystis microcysts have ranged from 34-100% in sheep under 1 year old. In this study, the prevalence of microcysts also no significantly increased with age (Fig. 1).

The observations of some studies performed on ovine sarcocystosis have shown that no significant difference was found in prevalence between both sexes of sheep (Martinez-Moreno *et al*., 1989; Seneviratne *et al*., 1975). The prevalence of Sarcocystis species recorded in this study was indicate similar study ($p<0.05$) (Fig. 3).

The results of many surveys (Kudi *et al*., 1991; Saito *et al*., 1996; Schmidtova and Jurasek, 1992; Savini *et al*., 1993) on ovine Sarcocystis species have revealed that sheep are infected by either only one or both Sarcocystis species forming microcysts in the muscles as in the present study.

The results of the present study have revealed that the prevalence of pathogenic ovine *Sarcocystis* species in Iran was 92%. In addition, these results points out the reality that the environment is heavily contaminated with sporocysts. Therefore, it has of great importance the farmers to be trained not to feed their dogs and cats with uncooked meat and the abattoir remnants to be burned in order to be effectively broken of infection cycle between the intermediate and the definitive hosts in Iran. The high rate of microscopic sarcocyst in sheep showed the high rate of dogs' sprocyst in pasture so to prevent infection of food animals they must be prevented from ingesting the sprocyst stage from dogs' feces in contaminated water, feed and bedding. These measures will prevent the development of intestinal stages where humans might serve as definitive hosts. Dogs are known as definitive hosts for some of the microscopic species of Sarcocystis (Dubna *et al*., 2007).

CONCLUSION

Results of this study showed a high frequency of Sarcocystis species infection in sheep slaughtered in the Isfahan, Qom and Shahre-Kord, Iran. Recently, additional diagnostic effort such as electron microscopy and molecular techniques has been employed for differentiation of various species of Sarcocyst in sheep. In this study, researchers used the squeezing method and found it was simple and rapid.

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