The role of sheep in the epidemiology of *Sarcocystis* spp. in Tabriz area northwest of Iran

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Received: 4 January 2014 / Accepted: 20 May 2014 / Published online: 1 June 2014
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Abstract The parasites of genus *Sarcocystis* are among the most commonly found parasites in domestic ruminants and some species of *Sarcocystis* can generate important economic loss when causing clinical and subclinical disease. In the present study, three techniques—direct inspection, impression smears and digestion method—were used for the diagnosis of *Sarcocystis* infection in 130 slaughtered sheep in Tabriz abattoir from April 2013 to October 2013. A total of 620 slaughtered sheep were selected randomly and their oesophagus and diaphragm were inspected using naked eye examination. In the second stage, 130 carcasses out of the 620 which had no apparent infection were selected randomly and their meat investigated using two techniques: impression smear with staining and pepsin digestion. The percentage of macroscopic cysts found in the oesophagus and diaphragm muscle was 5.64 and 2.74 %, respectively, and microscopically, infection was found in 100 % of the organs. There was no significant difference between different ages or between males and females. Although all of the sheep were found to be infected with *Sarcocystis*, majority of the cysts were demonstrated microscopically. This suggests that meat should be cooked sufficiently, since a macroscopic inspection may not provide true results. Also, 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 Tabriz city, northwest of Iran.

Keywords *Sarcocystis* · Sheep · Impression smear · Digestion method · Tabriz

Introduction

Sarcocystis species are widely distributed in a variety of mammals and birds (Neva and Brown 1994). *Sarcocystis* is one of the most prevalent protozoan parasites in the striated muscles of livestock slaughtered for food such as cattle, sheep and goat. Many reports on the prevalence of ovine *Sarcocystis* species have revealed that sarcosporidiosis is still an extremely common infection even in developed countries (Savini et al. 1992). The parasites of genus *Sarcocystis* are among the most commonly found parasites in domestic ruminants and some species of *Sarcocystis* can generate important economic loss when causing clinical and subclinical disease (Mitchell 1988). Pigs with heavy infections of 40 *Sarcocystis* or more per gram of diaphragm showed symptoms of weakness of loins, muscular stiffness, and temporary posterior paralysis. Lesions include enlargement and paleness of kidneys and inflammations of the mucosa of the stomach and intestine. Heart, diaphragm, and skeletal muscles are the preferred organs for *Sarcocystis* spp. location in the intermediate host and can persist through life in the hosts but many start to disappear after three months of inoculation (Mitchell 1988). Meat that is heavily infected may be condemned as unfit for human consumption. Eating raw or undercooked beef and pork containing mature sarcocysts of *S. hominis* and *S. suis* has resulted in humans acquiring intestinal sarcocystosis (Fayer 2004). As Tabriz city is one of the important territories for training domesticated animals, the aim of this study was to determine the prevalence of *Sarcocystis* infection in sheep in this region.

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Materials and methods

From April 2013 to October 2013, out of all the slaughtered sheep for human consumption, 620 sheep were randomly investigated for macroscopic cyst in a Tabriz abattoir, Tabriz, Iran, and 130 of the 620 carcasses, which with no apparent infection were selected randomly and the meat was inspected using two techniques: impression smear with staining and pepsin digestion. The animals included 73 males and 57 females, and their ages ranged from less than 1 to over 3 years of age. The investigated sheep were divided into groups according to age, sex and breed. Ages of the investigated sheep were assessed by visual inspection of teeth. The oesophagus and diaphragm muscles were chosen for inspection and sampling. Immediately after the animals were slaughtered, direct naked eye observation was made in order to detect macroscopic sarcocysts, while for the microscopic *Sarcocystis* spp. the following procedures were used:

1. Impression smear; approximately 1 g of muscle was cut into small pieces, approximately 3–5 mm thick, and crushed strongly between two glass slides and, after staining with Giemsa, examined under the microscope (400×).
2. Peptic digestion; approximately 20 g of muscle were crushed and digested for 60 min at 37 °C in 50 ml of digestion medium (containing 1.3 g pepsin, 3.5 ml HCl, and 2.5 g NaCl in 500 ml of distilled water). The sediment was then examined microscopically at 400× magnification.

Data were analyzed by SPSS software (version 16) and Chi square test analysis. The significance level was $P < 0.05$.

Results

Twenty-one of the 620 sheep (3.3 %) were diagnosed as being infected with macroscopic cysts. The prevalence of

Fig. 1 Microscopic cyst of *Sarcocystis* spp.

Fig. 2 Bradyzoites of *Sarcocystis* spp.

Table 1 Prevalence of macroscopic sarcocysts in different tissue and different sex and age groups of slaughtered sheep

Sex	Age (years)	No.	Esophagus	Diaphragm
Male	<1	76	4 (5.26 %)	2 (2.63 %)
	1–3	285	14 (4.91 %)	4 (1.40 %)
	>3	34	8 (23.52 %)	2 (5.88 %)
Female	<1	20	2 (10 %)	2 (10 %)
	1–3	138	4 (2.89 %)	3 (2.17 %)
	>3	67	3 (4.47 %)	4 (5.97 %)

infection in the oesophagus was higher than that of the other organs ($P < 0.05$). The infection rate increased with age, although this increase was not significant ($P > 0.05$). The infection rate was independent of sex (3.79 % in males and 2.66 % in females, $P > 0.05$). All microscopic samples were positive by the impression smear method and digestion method (Figs. 1, 2). There were no significant differences among the infection rates of the different organs ($P > 0.05$). Prevalence rates of macroscopic sarcocysts in different tissue and different sex and age groups of slaughtered sheep are presented in Table 1.

Discussion

Sarcocystis ovifelis, a macroscopic sarcocyst the most often identified from sheep, is of a worldwide distribution (Abo-Shehada 1996; Britt and Baker 1990; Dubey et al. 1988; Martinez Moreno et al. 1989), whereas *S. medusiformis*, another ovine macrocyst, has so far been reported only

from several countries (Abo-Shehada 1996; Collins et al. 1979; Oryan et al. 1996). In the present study, the oesophagus and diaphragm muscles were used, as previous investigations have shown these organs to be the most common sites for *Sarcocystis* infection. Results of this study showed a high frequency of microscopic *Sarcocystis* infection in slaughtered sheep in Tabriz, Iran. The high prevalence rate of microscopic sarcocysts (100 %) in sheep shows the importance of the infection for other intermediate host. There are a few reports on the prevalence of *Sarcocystis* in domestic animals in Iran, where the rate of macroscopic infection in sheep and goats in Khoram-Abad has been reported as (6.67 %) and (12.25 %) respectively (Atashparvar et al. 2001); and macroscopic prevalence rate of 3.58 % in sheep and 13 % in goats has been reported in Kerman (Radfar and Khosravi 2001), Fars (57.7 % in sheep) (Oryan et al. 1996). The prevalence of *Sarcocystis* spp. was 83.6 % by gross and histopathological examinations in slaughtered camels in the Mashhad Slaughterhouse, Eastern Iran (Valinezhad et al. 2008). The rate of infection reached 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 (Moré et al. 2008). In Brazil, all 50 samples of raw beef prepared for kibbe in 25 Arabian restaurants in Sao Paulo contained sarcocysts (Pena et al. 2001). The microscopic prevalence rate of *Sarcocystis* in other countries reported as follows: France (sheep 94.8 %), Ethiopia (sheep 93 %), Slovakia (sheep 87.6 %), Turkey (sheep 90 %), Iraq (sheep 97 %), Mongolia (sheep 96.9 %, cattle 90.0 %, camel 100 %) (Fukuyo et al. 2002; Latif et al. 1999; Ozturk 1994; Woldeemeskel and Gebreab 1996). The infection has potential public health importance because man may acquire infection by consumption of undercooked meat from infected cattle and pigs. Human consumption of raw or undercooked beef and pork containing mature sarcocysts of *S. hominis* and *S. suis*, respectively, can cause intestinal Sarcocystosis (Bunyaratvej et al. 2007). Dogs are known as definitive hosts for some of the microscopic species of *Sarcocystis* (Dubná et al. 2007). Recently, additional diagnostic efforts such as electron microscopy and molecular techniques have been employed for differentiation of various species of sarcocyst in sheep; for example, use of transmission electron microscope for differentiation of *S. tenella* and *S. arieticanis* in sheep based on cyst wall ultra-structure and thickness of cysts. Thin-walled cysts are *S. eariticanis* and thick-walled are *S. tenella* (Khalifa et al. 2008). Histological methods and PCR have also been employed to determine the regional prevalence and identity of *Sarcocystis* spp. in retail beef samples from the Americas (Pritt et al. 2008). They have also found that PCR assays may increase the detection

sensitivity of *Sarcocystis* spp. and contribute to diagnostic precision. A PCR technique has also been used to differentiate *S. hominis* in Belgian minced beef (Vangeel et al. 2007). Because of the consumption of raw minced beef, these findings may point to an underestimated risk to public health. Notably, little variability among *S. tenella* from a wide geographic range from the Americas or Asia has been identified (Rosenthal et al. 2008), and speculation exists on the role of sheep (and canine) domestication in constraining diversity in such parasites. Animals infected with sarcocystosis suffer from loss of weight, reduced milk production, anemia, abortion and death in severe cases. The high frequency of microscopic sarcocyst infection in sheep is associated with the fact that the sheep are in close association with canine sporocysts. The low prevalence of macroscopic sarcocysts (3.3 %) in the present study, compared with the prevalence of microscopic sarcocysts (100 %) may be due to this fact that such cysts are of feline origin, and in Tabriz contact between sheep and dogs in pasture is greater than between sheep and cats. It has been reported that muscle digestion is more sensitive than tissue sectioning in the detection of *Sarcocystis* infection (Savini et al. 1992). There are many species of *Sarcocystis* that infect dogs and cats, but none are pathogenic. As a result, no treatment is necessary but infection can be prevented by eating only well-cooked meat. Also, 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 Tabriz city.

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