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Survey on Sarcocystis Infection in Slaughtered Cattle in South-West of Iran, Emphasized on Evaluation of Muscle Squash in Comparison with Digestion Method

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Abstract: Sarcocystosis is a zoonotic disease in domestic animals caused by *Sarcocystis* sp., a cyst-forming coccidian parasite with obligatory two host life cycle involving carnivorous as definitive hosts and herbivorous or omnivorous as intermediate hosts. In present study muscles from skeleton, oesophagus, heart, tongue, diaphragm and abdomen from 344 cattle (176 male and 168 female), slaughtered from February 2009-October 2009 in Ahvaz, Khouzestan, South-West of Iran, investigated for either macroscopic or microscopic sarcocysts using naked eye examination for sarcocysts, peptic digestion and muscle squash for detecting bradyzoites. The prevalence of Sarcocystis infection was identified in 100% of cattle by digestion method and 94.7% by Muscle squash and just one cattle was infected with macroscopic cysts. The results showed that digestion method is a perfect method for diagnosing the sarcocysts in cattle.

Key words: Sarcocystis, cattle, digestion method, muscle squash, omnivorus, Iran

INTRODUCTION

Sarcocystis is one of the most prevalent parasites of the livestock that causes sarcocystosis. Sarcocystosis is an extremely common zoonotic infection even in the most developed countries of the world. This coccidian parasite causes intestinal and muscular sarcocystosis in immunocompetent patients (Velasquez *et al*., 2008). *Sarcocystis* sp. (Protozoa: Apicomplexa) have an obligatory two host (predator-prey) life cycle (Dubey *et al*., 1989a). Cattle become infected with the parasite often by ingesting tetrazoite sporocysts from contaminated food or water. Each intermediate and definitive host may harbour >1 *Sarcocystis* species (Fayer, 2004). Out of *Sarcocystis* species infecting domestic animals, a given species usually infects only one species of intermediate host and uses either felids or canids but not both as its definitive host. Infections can result in loss of weight, poor feeding efficiency, anorexia, fever, anaemia, muscle weakness, reduced milk yield, abortion and death in cases of very severe infection depends on the dose of ingested sporocysts and the immune status of the host (Dubey *et al*., 1989a). There are three species of *Sarcocystis* in cattle: *Sarcocystis cruzi*, *Sarcocystis hirsuta* and *Sarcocystis hominis*. *Sarcocystis cruzi* is the most common and important species affecting cattle (Heydorn *et al*., 1975; Mowafy, 2003). The main objective of this study was to determine the prevalence of Sarcocystis infection in Ahvaz, Kouzestan Province in south-west of Iran by using peptic digestion method and muscle squash methods and to measure the sensitivity of muscle squash in comparison with Digestion method as a gold standard.

MATERIALS AND METHODS

Sample collection: During February 2009-October 2009, 344 cattle included 176 males and 168 females ranged from 6 months 6 years old slaughtered in main abattoir of Ahvaz, center of Khouzestan Province in Iran were investigated for the presence of Sarcocystis cysts as well as bradyzoites in muscular tissues. The cattle were classified into 3 groups according to the age (under 2 years, 2-4 years and 4-6 years) by visual inspection of teeth. After direct observation by naked eye examination of slaughtered cattle for detecting macroscopic Sarcocysts at least 50 g of oesophagus, heart, tongue, abdomen and skeletal muscles collected and transferred to parasitology laboratory for microscopic examinations.

Digestion method: The method of Dubey *et al*. (1989a) with some modifications was used for digestion of muscles as described; 50 g of each collected muscles were minced and digested for 30 min at 37°C in 100 mL of

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digestion medium containing 1.3 g of pepsin (Merck), 3.5 mL and 2.5 g NaCl in 500 mL of distilled water. After digestion, the mixture were centrifuged 5 min at 1500×g and the sediment was then stained with giemsa and examined by optical microscope at 400× magnification for detecting bradyzoites.

Muscle squash: About 1 g of each collected muscle was cut into small pieces approximately 5 mm thick and crushed strongly between two slides. One of the slides fixed with methanol and stained by giemsa then examined with the optical microscope for bradyzoites at 400×.

RESULTS AND DISCUSSION

Out of 344 investigated cattle, macroscopic cysts were seen in just one high infected carcass and sarcocysts existed in all of the inspected muscles. The length of sarcocysts ranged from 4-9 mm. The overall prevalence of Sarcocystis infection in different examined muscles of 344 cattle based on detecting of bradyzoites was 100% by peptic digestion (Table 1). Muscle squash showed 94.7% of infection in cattle (326 of 344) including 93.3% (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 esophagus, 80.2% (276 of 344) in abdomen and 87.2% (300 of 344) in skeleton muscle (Table 1). Comparing with digestion method the sensitivity of muscle squash was 93.3, 87.5, 87.2, 85.1, 83.7 and 80.2% in heart, diaphragm, skeleton, esophagus, tongue and abdomen muscles, respectively.

Sarcocystiosis caused by different *Sarcocystis* species is a protozoal infection with worldwide distribution in many species of animals and human. Sarcocysts occurs either microscopic or macroscopic in striated muscles and sometimes in unstrained muscles (Dubey *et al*., 1989b). In the present study, the oesophagus, heart, tongue, diaphragm, abdominal and skeleton muscles were used as investigations have shown these organs to be the most common sites for Sarcocystis infection in cattle and some animals such as sheep, camel and buffaloe (Abo-Shehada, 1996; Beyazit *et al*., 2007; Latif *et al*., 1999).

High prevalence of Sarcocystis infection was seen in cattle slaughtered in Ahvaz. Bradyzoites were found in 100% of all different muscle samples of 344 cattle. This high prevalence identified by digestion method as a gold standard for diagnosis the bradyzoites in meat or other edible parts whereas numerous macrocysts have been identifed just in one cattle in all examined samples and in that infected cattle sarcocysts were distributed in all different muscles. About 100% rate of infection also reported by Fard *et al*. (2009) in south-east of Iran and Shekarforoush *et al*. (2006) in Fars Province, south of Iran. In the United States the rate of infection has been reported in 100% of cattle (Fayer and Dubey, 1986; Fayer, 2004). A high prevalence of infection was also reported in Argentina (More *et al*., 2008). In Iran the high prevalence of *Sarcocystis* sp. has been reported (83.6%) by gross and histopathological examinations in camels slaughtered in Mashhad, north-eastern of Iran (Valinezhad *et al*., 2008). Based on the hypothesis proposed by Farad *et al*. (2009), the high frequency of microscopic sarcocystis infection in cattle is associated with the fact that the cattle are in close association with dogs. The hypothesis is: it seems that this parasite is very successful organism in its relationship, interaction and adaptation with the host. For example in Khouzestan Province of Iran water buffaloes are also in close association with either dogs or cats. Nevertheless the prevalence of Sarcocystis infection in water buffaloes in this Province is relatively high (57% by peptic digestion and 54.6% by Elisa) (Ghorbanpoor *et al*., 2007) but it does not reach to the prevalence of Sarcocystis infection in cattle which recorded in different studies.

In the present study, the high prevalence rate of infections according to microscopic examination indicates the importance of the infection for the intermediate host and the probable contaminations of humans in Iran by Sarcocystis hominis. However, identification of species by accurate tools such as electron microscopy or

Table 1: Prevalence of *Sarcocystis* sp. infection based on digestion method and muscle squash in Ahvaz abattoir

Age		Esophagus		Heart		Diaphragm		Abdomen		Tongue		Total	
(year)	Number	D+*	S+**	D+	S+	D+	S+	D+	S+	D+	S+	D+	S+
2>	123	123 (100%)	103 (83.7%)	123 (100%)	116 (94.3%)	123 (100%)	105 (85.3%)	123 (100%)	98 (79.6%)	123 (100%)	100 (81.3%)	123 (100%)	119 (96.7%)
2-4	155	155 (100%)	134 (86.4%)	155 (100%)	143 (92.2%)	155 (100%)	137 (88.3%)	155 (100%)	124 (80%)	155 (100%)	129 (83.2%)	155 (100%)	144 (92.9%)
4-6	66	66 (100%)	56 (84.8%)	66 (100%)	62 (93.9%)	66 (100%)	59 (89.3%)	66 (100%)	54 (81.8%)	66 (100%)	57 (86.3%)	66 (100%)	63 (95.4%)
Total	344	344 (100%)	293 (85.1%)	344 (100%)	321 (93.3%)	344 (100%)	301 (87.5%)	344 (100%)	276 (80.2%)	344 (100%)	288 (83.7%)	344 (100%)	326 (94.7%)

*Number of positives in digestion method; **Number of positives in squash method

polymerase chain reaction is necessary. Electron microscopy and molecular techniques has been employed for differentiation of *Sarcocystis cruzi* and *Sarcocystis hominis* (Khalifa *et al*., 2008; Vangeel *et al*., 2007).

Human intestinal sarcocystosis is a zoonotic disease caused by two coccidians, i.e., *Sarcocystis hominis* due to consumption of raw infected beef and *Sarcocystis suihominis* due to consumption of infected raw pork. These species can cause digestive disturbances such as nausea, vomiting and diarrhoea (Dubey *et al*., 1989a). Various diagnostic methods such as pepsin or trypsin digestion, muscle squash, muscle squeezing and histopathological methods are used for the detection of sarcocystosis (Abo-Shehada, 1996; Beyazit *et al*., 2007; Latif *et al*., 1999; Sayed *et al*., 2008).

CONCLUSION

The researchers used the pepsin digestion and muscle squash examination methods and compared these two diagnostic methods. Both diagnostic methods had good sensitivities but we concluded that digestion method is more accurate.

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