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Prevalence of *Fasciola spp* infestation in slaughtered ruminants using by ELISA, fecal and macroscopic analysis in Ahvaz the capital of Khuzestan Province

Rahdar, M.[1,2]; Shadnoush, F[1,2]*.

1- Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2- Parasitology Department, Medical School, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

*Corresponding Author: shadnoush.5630@gmail.com

Abstract

Fasciolosis is a zoonotic parasitic disease with worldwide distribution. The disease causes significant economic losses in herd industry including decrease animal products such as meat, milk and infertility reduction. Human can acquire infection as accidental host especially in areas with high level of raining and humidity. Previous studies showed the prevalence of infection among meat-product animals based on inspiration of carcasses in abattoirs using macroscopic test. It seems that the inspiration evidence is not quite precise because the early infection cannot be recognized by macroscopic examination. Therefore, the present cross-sectional study was conducted to determine the Prevalence of fasciolosis on ruminants slaughtered at Ahvaz abattoir, Khuzestan province. Totally, 60 blood and fecal samples of sheep, 50 samples of cattle and 50 samples of buffalo were randomly collected through April to May 2016. Fecal samples were examined using concentration by flotation techniques for the presence of *Fasciola* ova. The sera from blood sample tested for the presence of anti *Fasciola spp* antibodies by ELISA technique and livers of slaughter animals were checked for presence of *Fasciola spp* infestation. In this study, Seroprevalence of fasciolosis was 5% for sheep, 20% for cattle and 18% for buffaloes. The incidence of liver fasciolosis in sheep, cattle and buffaloes samples were 6.6%, 18% and 12% respectively. Microscopic examination in fecal test was negative.

Keywords: Fasciolosis, Ruminants, Abattoir, ELISA.

Introduction

Fasciolosis is a parasitic disease that occurs worldwide and is caused by the digenetic trematodes called Fasciola *hepatica* and *F. gigantica*. The disease causes significant economic losses (Salimi-Bejestani et al., 2005). Fasciolosis reduces animal productivity and it causes secondary infections by decreasing immunity while the acute fasciolosis may lead to mortalities (Kamal, *et al*. 2016; Mason, 2004). It has also been recognized as one of the important problems in veterinary medicine (Kheirandish *et al.* 2016). Because of current changes in different climates conditions in the world, 2.4-17 million people are infected with *Fasciola spp* and 91.1 million people live in contaminated areas (Keiser & Utzinger, 2009). Iran is one of the endemic regions for Fasciolosis

and largest ever epidemics of fasciolosis have occurred in 1989 and the other in 1999 in north provinces of Iran (Moghaddam *et al.* 2004). Recently, the number of infected human has been raised so that thousands of infected individuals have been recorded in 42 countries of all continents from 1970-1990 (Chen & Mott, 1990). In Asia, human fasciolosis is mainly encountered in Iran and at a lower level in Vietnam. Iran is among the six countries, which are known to have a serious problem with fasciolosis (Organization, 2007). Fasciolosis usually manifests in two clinically phases; acute and chronic. The most important consequences of the disease are hepatic lesions, chronic inflammation of bile ducts and fibrosis. The major symptoms of the acute phase are fever, abdominal pain, gastrointestinal disturbances, urticaria, and respiratory symptoms (Ashrafi, 2015).

Several methods are used for the diagnosis of fasciolosis (Kheirandish *et al.* 2016). In the chronic phase, diagnosis is mainly carried out by detection of parasite ova in the stool or biliary aspirates but diagnosis by fecal examination is not possible during the 8–10 week pre-patent because the parasite is not mature at this time (Happich & Boray, 1969). Serological tests are used for detection of anti-Fasciola antibodies in serum samples in the acute phase and in ectopic areas. Immunological methods are also suitable for diagnosis of chronic fasciolosis by detecting specific antigens in stool samples and antibodies in the serum (Ashrafi, 2015), however, to improve diagnosis during both early and chronic phases of infection, several ELISA techniques have been described (Salimi-Bejestani et al., 2005). ELISA tests are very sensitive and specific (Saba et al., 2004). Therefore, the aim of the present study is to estimate exactly the prevalence of *Fasciola spp* infection in cattle, buffalo and sheep of the slaughterhouse of Ahvaz, in the city southwest of Iran.

Material and Methods

In this cross-sectional descriptive study, 60 blood and fecal samples of sheep, 50 samples of cattle and 50 samples of buffalo were randomly collected. A ten ml blood sample was taken from the jugular vein of each animal into evacuated tubes. The fecal samples were collected from the rectum of the animals and transported to the Parasitology Laboratory of the medical faculty, Jundishapur University of Ahvaz.

Sera were collected from the blood samples by centrifugation at 1500 rpm for 5 minutes, and stored at –20 ℃, until tested for the presence of anti-*Fasciola spp* antibodies by ELISA. Fecal samples were examined by flotation techniques for the presence of Fasciola eggs.

Standard flotation technique

Fecal samples were crushed and dissolved in saturated salt solution. After centrifugation, the supernatant was removed and the precipitate was added to a saturated solution. Then, a slurry was placed at the top of the tube and held for 20 minutes. After that, the coverslip was mounted and examined under the light microscope using objective of 10x to determine eggs (Benedeck, 1946).

Serological testing

The ELISA Kit (Pishtazteb) was used with a sensitivity of 92% and a specificity of 93%. At first, the samples were diluted with the aid of a diluent solution. Then, serum samples were added to the wells in duplicate and incubated for 30 minutes at room temperature. Both negative and positive control sera were included in each plate and were tested in duplicate. The plate was then washed with a washing solution. The conjugate solution was poured onto all the wells except for the blank well. Then the plate was incubated for 30 minutes at room temperature. The wells were washed again 5 times and the chromogen solution was added to the wells. The wells were incubated for 15 minutes at room temperature in the dark. In the last step, the stopping solution was added to each well, after which the absorbance of the wells was read at 450 nm. At the end, samples that light absorbance, high than Cut-off, are considered to be positive anti-fasciola antibodies and those lighting absorbance less than Cut-off, are considered to be negative anti-Fasciola antibody. According to the kit protocol, the following formula was used to calculate cut-off: Cut-off=0.25+Mean absorption of negative controls.

Samples preparation for postmortem inspection

Liver postmortem inspection by making multiple cuts and about 1 cm thick to check the presence of Fasciola (Soulsby, 1982).

Results

According to Table 1, the seroprevalence of fasciolosis, in this study, was found to be 5 %(3/60), 20 %(10/50) and 18 %(9/50) in sheep, cattle, and buffalo respectively. The incidence of liver fasciolosis in examined sheep, cattle and buffaloes samples were 6.6%, 18%, and 12% respectively. However, no fasciola egg was found egg in stool examination.

Table 1. The results of Fasciola spp infection in slaughtered animals in the province Khuzestan.

Livestock type	ELISA test	Liver inspection	Stool exam
Sheep	5%(3/60)	6.6%	0
Cattle	20%(10/50)	18%	0
Buffalo	18%(9/50)	12%	0

Discussion

Infection with *Fasciola hepatica* and *Fasciola gigantica* is found among cattle, sheep, goats, buffalo and some other animals such as horses and rabbits in most parts of the world, with rates as high as 90% among livestock in many countries (Salimi-Bejestani *et al.* 2005), Human Fasciolosis has been increased during the last four decades. The total number of people infected with fasciolosis reported from different countries until 2002 was estimated to be 830,000 in Egypt, 742,000 in Peru, 360,000 in Bolivia, 20,000 in Ecuador, and 37,000 in Yemen. In some countries such as Bolivia, prevalence was reported to be as high as 70% (Kooshan *et al.* 2010). A high rate of human infection in epidemic forms was recently reported in some countries including Iran and Eastern Anatolia in Turkey in 2008 (Akca *et al.* 2014). The prevalence rate of liver trematodes in ruminants is variable markedly worldwide. *Fasciola spp* are frequently found in herbivores in different parts with the various rate among sheep and goat as 7.1% and 3.9%, respectively (Nyindo & Lukambagire, 2015). In Iran, there had been reports of various fasciolosis rates in ruminants. Sabbaghian *et al.* (1964) demonstrated a high prevalence of infection in livestock of Guilan and Khuzestan Provinces respectively. Similarly, Movassagh showed that 8.57% of sheep livers were infected by *Fasciola hepatica* in the Northwest of Iran (Okajima *et al*. 2016). Kordshooli *et al*. (2017) observed that 25.9% of cattle, 5.3% of sheep, and 4.9% of goat were infected by *Fasciola spp* (Moreover, an abattoir survey in Ardabil showed that among liver flukes infections, fasciolosis was detected in 25.6% of cattle

and 21.6% of sheep (Ollerenshaw & Smith, 1969). In Pakistan, a considerably high prevalence rate of *F. hepatica* in cattle (85.1%), sheep (51.3%) and goats (14.8%) was reported (Rollinson, 1995). According to the slaughterhouse information, similar results were also reported in Kenya where a considerable rate of fasciolosis was seen in cattle (52.6%) compared to sheep (18.3%) and goat (16.9%) (Yusuf et al., 2016). In a 10 year abattoir investigation in Mazandaran Province, higher fasciolosis prevalence was observed among cattle, more than sheep (Massoud, 1990).

A study, conducted in Rudsar in north of Iran between 2011 and 2012, Radfar *et al*. presented that the prevalence of infection with fasciolosis among slaughtered cattle in the slaughterhouse was 20.14%, indicating a high prevalence of this infection in northern part of Iran which is due to favorable weather conditions for growth of intermediate snails (Radfar et al., 2015). According to Maleki's research, the prevalence of livestock contamination in Khuzestan was 82% and Islami (quoted by Changizi) reported this infection in Dezful in 1988 about 31%_____(Changizi et al., 2006) (Changizi, 2006). The findings of this study indicate that fasciolosis is highly prevalent in the province of Khuzestan. In a recent abattoir study undertaken in Dezful (2015), the level of fasciolosis was found to be around 10 %. Moreover, the proportion of false negative findings in this type of research should not be underestimated. The rate at which animals are slaughtered and the inappropriateness of the conditions under which examinations are carried out increase the likelihood that the presence of parasites may be overlooked, particularly in cases of mild infection. Likewise, the fact that the parasites are small and embedded in the parenchyma in the prepatent phase of the infection significantly reduces the probability of detecting the infective agent under abattoir conditions. Moreover, since animals sent to the abattoir have usually been treated with anti-parasite drugs and fattened prior to slaughter is taken into consideration, it becomes evident that the use of abattoir data is less than sufficient to determine accurately the level of prevalence in a region. But by using the ELISA technique, we can identify all animals that were at one stage of life with Fasciolosis. In a comparative study performed in Switzerland, Rapsch *et al.* (2006) found that while the level of sensitivity of abattoir results was 64%, in contrast, the diagnostic sensitivity of the ELISA test was 93% (Rapsch et al. 2006). Likewise, in a more recent study monitoring fasciolosis in dairy herds in Carinthia Austria, Duscher *et al*. (2011) found that both individual milk ELISAs and blood serum ELISAs are more sensitive

than fecal examination to fluke eggs and copra-antigen ELISA.

Conclusion

In conclusion, it is clear that infection with fasciolosis is widespread in cattle, buffalo, and sheep in Khuzestan because of the level exposure to the infection of farm animals in the area is very high. The development of an effective control strategy to present the infection and to diminish the loss of economic effects on the livestock industry is required.

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