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Prevalence and Intensity of *Dicrocoelium dendriticum* in Sheep and Goats of Iran

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ABSTRACT

Dicrocoeliosis is a hepatic parasitic disease of clinical and financial significance in ruminant breeding throughout the world. The present study was carried out to estimate the prevalence and intensity of *Dicrocoelium dendriticum* in slaughtered sheep and goats in various geographical areas of Iran. In a cross-sectional study, totally, 10190 of slaughtered sheep and goats at abattoirs located in 8 provinces of Iran were examined via necropsy of livers and gall bladders. Specimens were identified morphologically then the collected parasites were counted. Overall 0.93% of the livers were found to be infected. The prevalence of *Dicrocoelium* infection in sheep and goat were 0.85 and 1.29%, respectively. The highest prevalence in sheep and goats belong to East Azerbaijan province and the lowest belong to Fars province. This study indicated that the prevalence of dicrocoeliosis in sheep and goat of Iran was quite lower than expected.

Key words: Sheep, goat, *Dicrocoelium dendriticum*, prevalence, intensity, Iran

INTRODUCTION

Dicrocoeliosis, caused by *Dicrocoelium dendriticum* (Digenea, Dicrocoeliidae), is a hepatic parasitic disease of clinical and financial significance in ruminant breeding which causes direct losses due to confiscation of parasitized livers (Jithendran and Bhat, 1996) and indirect losses due to hepatobiliary alterations produced by the parasites and the costs associated with anthelmintic treatments (Otranto and Traversa, 2002; Manga-Gonzalez *et al.*, 2004; Ferreras-Estrada *et al.*, 2007). There are at least two species of *Dicrocoelium*, *D. dendriticum* and *D. hospes*, live in the bile ducts and gall bladder of domestic and wild ruminants and sporadically in humans, are the causative agents of dicrocoeliosis (Gutierrez, 2000). Dicrocoeliosis is believed to be endemic or potentially endemic in 30 countries. *D. dendriticum* (Rudolphi 1819), is occurs throughout Europe (former U.S.S.R., Switzerland, Italy, Germany, Spain, Turkey), the Middle East (Iran), Asia (China, Japan, Philippines, Syria, Vietnam), Africa (Ghana, Nigeria, Sierra Leone) and in North and South America and Australia. The prevalence of dicrocoeliosis is worldwide and is particularly epidemic in lowland or mountain pastures which provide adequate conditions for the survival and development of terrestrial snails and ants. The parasite tends to be found in areas that favor the intermediate hosts, such as fields with dry, chalky and alkaline soils (Gideon, 2009). In 2007, the World Health Organization included *D. dendriticum* on its list of organisms

to target with its Food borne Disease Burden Epidemiology Reference Group (WHO, 2007). In Iran dicrocoeliosis by *D. dendriticum* is a widespread trematode infection. Previous epidemiological studies on sheep and goat showed a high rate of *D. dendriticum* infection in the definitive host range from 6.8 to 23.25% (Daryani *et al.*, 2006; Ahmadi *et al.*, 2010). Although, the main enzootic area of sheep dicrocoeliosis have been reported in the north of country and the parasite also occur in the various geographical regions (Daryani *et al.*, 2006; Ahmadi *et al.*, 2010; Ahmadi and Meshkekar, 2010; Ansari-Lari and Moazzeni, 2006). There is a strong need for better epidemiological knowledge of the infection in sheep in order to establish correct strategic control measures . Since, there is a high concentration of pastured livestock on traditional forms and there was no comprehensive statistically sound and epidemiologic data about *D. dendriticum*, so, the present study was performed to estimate the prevalence and intensity of liver fluke infections in abattoir populations of sheep and goats in various geographical areas of Iran for the period 2010-2011.

MATERIALS AND METHODS

The study was performed in a cross-sectional field in eight provinces (East Azerbaijan, Markazi, Isfahan, Khuzestan, Mazandaran, Fars, Kherson Razavi, Tehran) of Iran (Fig. 1) from 20 March 2010 to 19 March 2011.

Specimens: Totally, 10190 of slaughtered sheep and goats were examined via necropsy of livers and gall bladders. Specimens were identified morphologically, then the collected parasites were counted (Yamaguti, 1971; Taira *et al.*, 2006).

Histopathology study: Tissue samples were taken from each infected livers. The tissue samples were fixed in 10% buffered formalin and embedded in paraffin wax. Sections 4 μ m thick were stained with haematoxylin and eosin (H, E)a for histopathological study.



Fig. 1: Map of the study area showing the location of provinces

Statistical analyses: To compare and interpret the data obtained, One-way analysis of variance, lowest significant difference between pairs of mean value (LSD) test was used. The statistical analyses were carried out using the SPSS software (version 16).

Intensity Rate was calculated by formula:

$$\text{Intensity rate} = \frac{\text{Total No. of collected parasites}}{\text{No. of infected animals}}$$

RESULTS AND DISCUSSION

A total of 10190 livers including 8170 from sheep and 2020 from goats were examined and overall 0.93% of the livers were found to be infected (Table 1). The prevalence of *Dicrocoelium* infection in sheep and goat were 0.85 and 1.29%, respectively. The highest prevalence in sheep and goats belong to East Azerbaijan province (2.50 and 6.67%, respectively) and the lowest belong to Fars province (Table 1).

The infection rate in female sheep was more than males and the infection rate in female goats was lower than males ($p > 0.05$). The highest mean number of parasite was found in Razavi Khorasan sheep and Mazandaran goats. The lowest mean number was in Khuzestan sheep and Fas goats (Table 2). The intensity rate of infection, in sheep was higher than goats where in male sheep and goats the rate was higher than female ($p < 0.001$) (Table 3, 4).

Table 1: Prevalence of *D. dendriticum* in sheep and goat slaughtered in various provinces of Iran

Province	Sheep			Goat			Total		
	No. examined	No. of infected liver	Infected liver (%)	No. examined	No. of infected liver	Infected liver (%)	No. examined	No. of infected liver	Infected liver (%)
Markazi	350	3	0.86	150	4	2.67	500	7	1.40
Isfahan	500	6	1.2	250	5	2	750	11	1.47
Khuzestan	400	2	0.5	50	1	2	450	3	0.67
East Azerbaijan	720	18	2.5	60	4	6.67	780	22	2.82
Mazandaran	500	12	2.4	270	5	1.85	770	17	2.21
Fars	950	4	0.42	850	3	0.35	1800	7	0.39
Razavi Khorasan	3900	20	0.51	290	3	1.03	4190	23	0.55
Tehran	850	4	0.47	100	1	1	950	5	0.53
Total	8170	69	0.85	2020	26	1.29	10190	95	0.93

Table 2: Number of *D. dendriticum* collected from sheep and goats of various region of Iran

Province	No. of parasites in sheep			No. of parasites in goat		
	Mean±SE	Min.	Max.	Mean±SE	Min.	Max.
Markazi	180.00	-	180	137.50±53.75	20	270
Isfahan	165.11±42.71	35	400	52.78±10.50	15	115
Khuzestan	120.50±19.50	101	140	474.00	-	474
East Azerbaijan	203.60±35.88	63	395	310.00±90.44	70	475
Mazandaran	1450.00±304.14	950	2000	582.33±164.86	90	1400
Fars	845.33±485.82	86	1750	50.00±20.00	30	70
Razavi Khorasan	982.67±273.94	25	4000	130.00±120.00	10	250
Tehran	673.75±318.56	150	1510	135.00	-	135
Total	601.36±113.13	25	4000	264.84±61.085	10	1400

Table 3: Intensity rate of *D. dendriticum* collected from male and female sheep slaughtered in various provinces of Iran

Province	In male sheep		In female sheep		Total	
	Total No. of collected parasites	Intensity rate	Total No. of collected parasites	Intensity rate	Total No. of collected parasites	Intensity rate
Markazi	180	180.0	90	45.0	270	90.0
Isfahan	476	158.7	1010	336.7	1486	247.7
Khuzestan	140	140.0	101	101.0	241	120.5
East Azerbaijan	1324	132.4	712	89.0	2036	113.11
Mazanderan	0	0.0	4350	362.5	4350	362.5
Fars	2536	634.0	0	0.0	2536	634.0
Razavi Khorasan	13480	898.7	1260	252.0	14740	737.0
Tehran	2545	848.3	150	150.0	2695	673.75
Total	20681	558.9	7673	239.8	28354	410.92

Intensity rate = Total number of collected parasites/Number of infected animals

Table 4: Intensity rate of *Dicrocoelium dendriticum* collected from male and female goats slaughtered in various provinces of Iran

Province	In male sheep		In female sheep		Total	
	Total No. of collected parasites	Intensity rate	Total No. of collected parasites	Intensity rate	Total No. of collected parasites	Intensity rate
Markazi	460	115.0	0	0.0	460	115.0
Isfahan	285	95.0	190	95.0	475	95.0
Khuzestan	0	0.0	474	474.0	474	474.0
East Azerbaijan	806	268.7	434	434.0	1240	310.0
Mazanderan	4991	1247.8	250	250.0	5241	1048.2
Fars	100	33.3	0	0.0	100	33.3
Razavi Khorasan	250	125.0	10	10.0	260	86.7
Tehran	135	135.0	0	0.0	135	135.0
Total	7027	351.4	1358	226.3	8385	322.5

Intensity rate = Total number of collected parasites/Number of infected animals

In gross feature of infected livers, obstruction of bile duct, indurate and markedly distended liver with severe fibrosis were observed (Fig. 2). Fibroplasias and cellular infiltration accompanied by granulomas in the parenchyma beneath the partial surface were considered as the major changes and histopathological effect of *D. dendriticum* in infected livers (Fig. 3, 4).

The environmental and ecological aspects are the main important factors for *Dicrocoelium* life cycle between intermediate and definitive hosts. This situation is available in the most provinces of Iran. Although, the highest and lowest prevalence are belong to East Azarbaijan and Fars but the difference of the rates is not significant. Both provinces are mountains and sheep and goats are rearing in traditional form in mountain pasture. The weather of East Azarbaijan is cold and Fars is relatively dry. Dicrocoeliosis occurs in large lowland or mountain pasture areas which provide suitable conditions for the survival and development of the individual species of terrestrial snails and ants (Eckert and Hertzberg, 1994). Intermediate hosts do not require damp surroundings and they can therefore occur in pasture land diffusely. The disease occurs mainly in drier regions with limestone subsoil, however, attention to the fact that dicrocoeliosis is not only limited to



Fig. 2: Abnormal liver infected with *D. dendriticum*; obstruction of bile duct, indurate and markedly distended liver with severe fibrosis

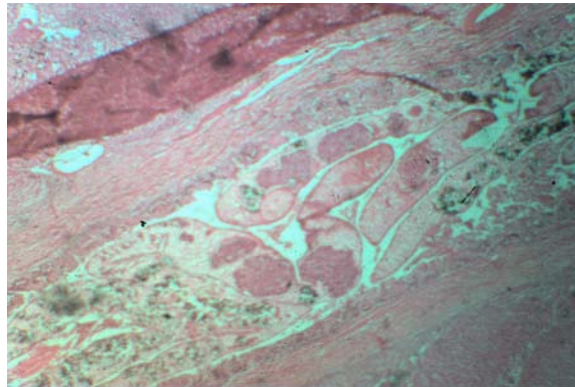


Fig. 3: Major changes in the livers and histopathological effect of infected with *D. dendriticum*: fibroplasias and cellular infiltration accompanied by granulomas in the parenchyma beneath the partial surface

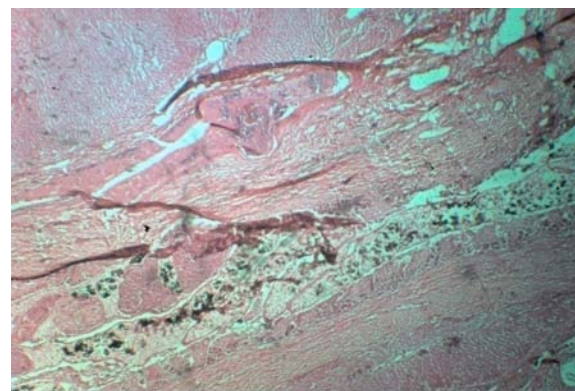


Fig. 4: Adult worm in a septal bile duct. Hyperplasia of the bile duct in a sheep liver infected with *D. dendriticum* (H, E)

mountainous limestone regions and it also occurs in lowlands with a small amount of limestone in the brown-soil area. Mountain pastures are contaminated with the eggs of fluke mainly from infected domestic ruminants and wild ruminants can also contribute to the spread of infection. It is the wildlife species infected with flukes that disseminates the germs also in the pastures shared with farm animals, thus becoming a significant factor in the maintenance of the localities of the disease and overall spread of dicrocoeliosis (Duchaek and Lamka, 2003). The eggs of *D. dendriticum* are capable of surviving in dry pastures for more than one year (Kassai and Bekesi, 1993). Fluke eggs may over-winter and remain infective for up to 20 months on pastures (Otranto and Traversa, 2002).

Dicrocoeliosis is believed to be endemic or potentially endemic in 30 countries and occurs in both pasture-bred and wildlife species throughout the world. The disease is common in those regions of Europe, the Middle East, Asia, North Africa and in North and South America and Australia, where the local conditions are favorable for certain species of earth snails and ants as intermediate hosts (Gideon, 2009). In Iran, dicrocoeliosis occurs in the regions with pasture breeding of ruminants and its prevalence does not exceed 25% (Daryani *et al.*, 2006; Ahmadi and Meshkehkar, 2010). In the present study, *D. dendriticum* was found in 0.93% of liver sheep and goats. On the other hand the mean prevalence of *D. dendriticum* in sheep and goats was 0.85 and 1.29%, respectively. Although, the difference between the rates is not significant but the epidemiologic implication of this finding might be attributed at least partly to the sources of their main food. Main food of sheep is herbs of mountains and plains, while goats are mainly feed with leaf of tree. Some other studies which were carried out in Iran in the last decade, indicating variable prevalence rates of *D. dendriticum* in different regions of country. The range of prevalence is from 0.22 to 20%. In a study in Ardabil Province (North Iran), *D. dendriticum* was reported in sheep and goats 5.3 and 4.9%, respectively (Daryani *et al.*, 2006). In Oryan *et al.* (2011) study in Northeast of Iran, the infection rate of *D. dendriticum* was reported in sheep and goats 5.3 and 1.41%, respectively (Oryan *et al.*, 2011). In a study conducted by Ghazani *et al.* (2008) in the northwest region of Iran, 20% of sheep livers were infected with *D. dendriticum* (Ghazani *et al.*, 2008). According to Ansari-Lari and Moazzeni's study, prevalence rate of dicrocoeliosis was 0.80% in sheep in Shiraz (Fars Province) (Ansari-Lari and Moazzeni, 2006). Evaluation of liver parasite disease slaughterhouse in Kerman province showed that the infection rate of dicrocoeliosis in sheep was 0.26 percent (Ranjbar *et al.*, 2010). In a study conducted by Youssefi *et al.* (2010), the prevalence of *D. dendriticum* in sheep slaughtered in Babol city, was 38.2% (Youssefi *et al.*, 2010). However Iran is considered as an endemic region for *D. dendriticum* but during last 10 years due to successful anthelmintics program of veterinary organization the prevalence rate of *Dicrocoelium* has dropped considerably. The prevalence rates for this trematode in neighboring countries were compared to our results indicating that the rates were lower in our study. The infection rates of *D. dendriticum* in livestock in Turkey was changing from 3.0-55.6% (Kara *et al.*, 2009). In Saudi Arabia *D. dendriticum* is recorded only in imported sheep (Nasher, 1990). Among the parasitic diseases affecting ruminants, dicrocoeliosis is a little known disease that is often underestimated by researchers. This is mainly due to the fact that dicrocoeliosis is asymptomatic and masked by the pathological effects of multiple parasitic infections in ruminant livestock. The infection is not often apparent to the farmers but is of considerable economic and public health importance. It is difficult to determine clearly the pathogenic effects of dicrocoeliosis since it is difficult to produce the experimental infections required to define its pathogenesis. A direct correlation was observed between worm burden and lesion scores in infected animals and five different degrees of

macroscopic liver lesions were classified, from normal liver to indurated liver with scarring, markedly distended liver ducts thickened with severe fibrosis and heavy worm burden (Theodoridis *et al.*, 1991). Modifications of bile duct surfaces and fibrotic lesions of the liver were observed to increase with changes in the level of infection up to 300 *D. dendriticum* while, above this value up to 600 flukes, a decrease was observed, perhaps due to the hosts' reaction (Camara *et al.*, 1996). In heavy infections a large number of worms are detectable inside the bile ducts and gall bladder, the liver is swollen, with thickened ducts, cholangitis, whitish spots on the surface, marked scarring and cirrhosis which result in liver impairment (Jithendran and Bhat, 1996). In histopathological change of liver of infected animals, whitish dilated intrahepatic bile ducts were exposed on the visceral surface of the liver, mainly on the left hepatic lobe. However, the incised surface of the liver revealed numerous enlarged bile ducts with thickened walls containing some parasites and a yellowish fluid. Hyperplasia, desquamation and necrosis of the epithelial cell lining, goblet cell differentiation and the presence of intraepithelial globule leukocytes are the main histopathological alterations that observe in the septal bile ducts. Small and moderate fluke infections take place subclinically. Serious long-lasting infection (over 5000 trematodes in sheep) causes total induration and cicatrization of the liver with chronic cholangitidis, extension of the biliary ducts and fibrosis evaluated the role of the number of parasites in the development of changes induced by flukes in the bovine liver tissue. The changes in the surface biliary ducts and lesions of liver cirrhosis were increased with the intensity of infection from 0 to 300 of *D. dendriticum*. With the intensity of infection of 301-600 flukes, they observed a decrease in their further development (Jithendran and Bhat, 1996; Camara *et al.*, 1996).

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