DOI: 10.22059/ijvm.2017.236806.1004822 *Iranian Journal of Veterinary Medicine*

The prevalence and intensity rate of *Dicrocoelium dendriticum* infection in ruminants of 3 provinces in coastal regions of the Caspian Sea

Majidi-Rad, M.[1], Meshgi, B.[1*], Bokaie, S.[2]

[1]*Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
[2]*Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

Key words:
Dicrocoelium dendriticum, intensity, prevalence, sheep, trematode ruminant, cattle

Correspondence
Meshgi, B.
Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Tel: +98(21) 61117070
Fax: +98(21) 669333222
Email: bmeshgi@ut.ac.ir

Received: 4 July 2017
Accepted: 2 October 2017

Abstract:

BACKGROUND: Dicrocoeliasis is caused by digenean trematode of *Dicrocoelium dendriticum*, small liver fluke, a hepatic parasitic disease in ruminants and human, throughout the world. *D. dendriticum* infection has been considered to be correlated with the economic and veterinary aspects. **OBJECTIVES:** The aim of the present study was to determine the prevalence and intensity of infection with *D. dendriticum* in sheep and cattle in Guilan, Mazandaran and Golestan provinces from Caspian Sea Littoral, Northern part of Iran. **METHODS:** For this purpose, 4 cities in every province, 4 villages in each city, and 4 districts in each village were chosen for sampling. Totally, 1344 faecal samples of sheep and cattle were examined. It should be taken into consideration that the number of eggs per gram of feces (E.P.G) was determined by flotation technique. **RESULTS:** The results suggested that sheep were infected 4-5 times more than cattle in all three provinces. The average prevalence of *D. dendriticum* infection in sheep and cattle was 36.21% and 9.37%, respectively in Guilan province. The average prevalence in Mazandaran province was determined to be 21.35% and 4.16% in sheep and cattle, respectively. It is worth noting that the highest infection rates occurred in Chalus and Noor from Mazandaran province. The average of *D. dendriticum* infection was determined to be 6.87% in sheep and 1.87% in cattle from Golestan province. **CONCLUSIONS:** Our results indicated that not only the prevalence but also the intensity of infection was higher in sheep compared to cattle Furthermore, the rate of infection was about two-fold higher in both hosts from Guilan province. Therefore, according to the higher prevalence of infection in two provinces of Guilan and Mazandaran and the importance of sheep in distribution of infection, sever control programs are required by providing comprehensive plans.

Introduction

Dicrocoelium dendriticum is one of the digenean trematodes that primarily live in bile ducts of a wide range of herbivorous as final hosts. However, humans, carnivores

and rodents can be infected with the small liver trematode (Meshgi et al., 2012; Ohotori et al., 2014). The life cycle of Dicrocoelium has unique complexities, because it involves both snails and ants as first and second intermediate hosts. The main host will be affected by eating metacercaria-infected ants (Otranto and Traversa 2002).

Three known Dicrocoelium species include *D. hospes*, *D. chinensis* and *D. dendriticum*, the first species belongs to Africa, the latter is found in Asia (China) and the third is worldly distributed (Manga-Gonzalez et al., 2001; Otranto et al., 2007).

Although *D. dendriticum* is native in cattle of Iran, its different aspects have been neglected in the country. There have been very few epidemiological based studies in Iran. Most of the documents in Iran are abattoir-based reports on the prevalence of infection in sheep, goat and cattle. Different abattoir-based studies have been conducted in several provinces including Fars (Ansari-Lari, and Moazzeni, 2006), Guilan (Ahmadi et al., 2010), Northern Khorasan (Oryan et al., 2011) and Lorestan (Ezatpour et al., 2014). In a study, the prevalence of Dicrocoelium infection in dogs has been reported to be 14% in a region of Khorasan-Razavi province (Beiranvand et al., 2013).

Although serological methods have been developed for diagnosis of Dicrocoelium, the use of slaughterhouse examination (evaluation of adult worms in the liver) in this context seems to be more common at detecting dicrocoeliasis after stool examination compared with the other parasitological methods (Meshgi and Khodaveisi, 2014; Rehbin et al., 2002). It is worth noting that dicrocoeliasis is not only important in terms of health, but also in terms of economics. Confiscation of parasitized livers lead to direct losses and indirect losses due to hepatobiliary alterations which can affect on animal food digestion, growth, milk and meat production and then lead to heavy economic losses (Manga-Gonzalez et al., 2004; Manga-Gonzalez et al., 2010).

Presence of a wide range of hosts, as well as a variety of land snails (100 species) and ants (21 species) as intermediate host have a great impact on the prevalence of Dicrocoelium infection in endemic areas (Manga-Gonzalez et al., 2001). Although Dicrocoelium infection has been present in Iran since ancient times, its prevalence has been increasing in recent years. However, there is not any report or other scientific documents about this fluke infection based on stool test (in live animals). Therefore, the present study was aimed to estimate the prevalence and intensity of Dicrocoelium infection in sheep and cattle in littoral states of the Caspian Sea.

Material and Methods

Sampling sites: The present study was conducted in Guilan, Mazandaran and Golestan provinces in the coastal strip of the Caspian Sea.

In total, 1344 faecal samples were collected directly from the rectum of 672 cattle and 672 sheep over a period of 3 months in the fall of 2016. For this purpose, 4 cities in every province, 4 villages in each city, and 4 districts in each village were chosen for sampling. Finally, a total of 48 geographic areas were sampled (Table 1).

Stool examination: Samples were collected directly from the rectum of all animals. Stool samples (4-6 gr) were labelled immediately (location name, collecting date, and kind of animal, etc.) and separate-

Table 1. The number of collected samples based on geographic location and type of livestock. * Different Villages = A, B, C, D.

Locality			No. of Sample		Total
Province	City	Villages*	Sheep	Cattle	
Guilan	Amlash	A, B, C, D	20×4	20×4	320 2
	Langrood	A, B, C, D	20×4	20×4	
	Talesh	A, B, C, D	20×4	20×4	
	Rood Bar	A, B, C, D	20×4	20×4	
Mazendran	Chalus	A, B, C, D	12×4	20×4	192 2
	Noor	A, B, C, D	20×4	20×4	
	Amol	A, B, C, D	20×4	20×4	
	Sari	A, B, C, D	20×4	20×4	
Golestan	Bandar-Gaz	A, B, C, D	10×4	10×4	160 2
	Aliabad-katul	A, B, C, D	10×4	10×4	
	Gonabad	A, B, C, D	10×4	10×4	
	Maravetappe	A, B, C, D	10×4	10×4	
Total	48	672	672	1344	

ly transferred to the parasitology laboratory.

The samples (4-6 gr) were examined using flotation method by Zinc chloride and sodium chloride saturated solution (specific gravity: 1.58). The number of Dicrocoelium eggs per gram of feces (EPG) was obtained based on the type of livestock and sampling area.

Results

The present study was aimed to determine the prevalence and intensity of sheep and cattle Dicrocoeliasis in the province of Guilan, Mazandaran and Golestan along the Caspian Sea. Moreover, the results are presented in two parts: the frequency and intensity of infection.

The prevalence of infection: Our finding indicated that sheep was infected 4 times more than cattle in all three provinces.

The average prevalence in Guilan Province was 36.21% in sheep and 9.37% in cattle. The highest infection rates in both hosts belonged to Langrood and Talesh (about 50% of sheep and 10% - 12.5% of cattle).

The average prevalence in Mazandaran province was about half that of infection in Guilan province. As matter of fact, it was calculated to be 21.35% and 4.16% in sheep and cattle, respectively. Furthermore, the highest infection rates were observed in Chalus and Noor, Mazandaran province (about 23%-33% in sheep and 6%-10% in cattle). In Golestan province, infection of sheep was only observed in Aliabad-Katul and Bandar-Gaz. The average infection of sheep and cattle was determined to be 6.87% and 1.87% in Golestan province (Table 2).

The intensity of infection: The average number of eggs per gram of feces in sheep was higher compared with cattle. The average number of Dicrocoelium eggs per gram of feces in sheep in three provinces of Guilan, Mazandaran and Golestan was 20.81, 6.76 and 2.44, while it was 6.86, 2.28 and 1.5 in cattle, respectively. Significant difference was observed between the EPG in different areas, but the highest rates were obtained in Langrood (about 32 in sheep and 9 in cattle) and Talesh (about 27 in sheep and 9 in cattle) from Guilan province (Table 2).

Table 2. Prevalence and intensity of Dicrocoelium infection in 3 provinces, Coast-

Table 2. Prevalence and intensity of Dicrocoelium infection in 3 provinces, Coastal regions of the Caspian Sea in the present study.

Locality		Dicrocoelium infection			
		Sheep		Cattle	
Province	City	(%)	(EPG)	(%)	(EPG)
Guilan	Amlash	16.25	11.92	6.25	4.2
	Langrood	51.25	20.21	12.5	6.75
	Talesh	51.25	27.04	10	8.74
	Rood Bar	36.25	12.24	7.5	5.33
Total		36.21	20.81	9.37	6.86
Mazendran	Chalus	22.92	8.1	10.42	5.8
	Noor	33.34	8.9	6.25	3.34
	Amol	16.66	6.75	0	0
	Sari	12.5	3.34	0	0
Total		21.35	6.76	4.16	2.28
Gloestan	Bandar-Gaz	7.5	6.67	0	0
	Aliabad-katul	20	3.12	5	2
	Gonabad	0	0	0	0
	Maravetappe	0	0	2.5	4
Total		6.87	2.44	1.87	1.5

al regions of the Caspian Sea in the present study.

Discussion

This study is a comprehensive survey of *D. dendriticum* infection in 3 provinces of the Caspian Sea, northern part of Iran. A total of 1344 fecal samples of sheep and cattle were collected and then tested from different climatic zones in Guilan (n=640), Mazandaran (n=384) and Golestan (n=320).

The status of Dicrocoelium infection in three provinces has shown a significant difference. The average prevalence of infection in sheep and cattle from Guilan was 36.21% and 9.37%, while it was 21.35% and 4.16% in sheep and cattle of Mazandaran province, respectively. Furthermore, infection in Golestan province was at a lower level than the other two provinces (in sheep 6.87% and cattle 1.87%).

It should be taken into consideration that *D. dendriticum* is important for two reasons. First, the zoonotic potential of *D. dendriticum*. Transmission pattern of infection in definitive hosts, including humans and animals, is by eating ants infected with metacercaria. In this regard, human is known as an accidental host, when infected ant is eaten accidentally by human via food (such as bread or vegetables). However, human infections are generally rare but about other hosts, especially ruminants, presence of ants in pastures and meadows greatly increase the possibility of livestock infection such as cattle and sheep (Fakour and Meshgi, 2011; Kose et al., 2015).

The second importance of Dicrocoelium infection in large and small ruminants is due to severe economic losses. On the one hand, confiscation of parasitized livers in the slaughterhouse inspection leads to direct losses, weight loss, reduced milk, anemia, reduced product quality, digestive disorders, liver failure, and other factors can cause indirect economic losses (Mauelli et al., 2007). It should be noted that although

indirect loss is less measurable, but due to its gradual, progressive and non-visible nature, it can have very important consequences.

Previous studies in Iran are based on the slaughterhouse reports and just according to the confiscation of parasitized livers. This method has great weaknesses for estimating the true prevalence. For example, the native nature of infection is not evaluated in this method, or cases of slaughtered animals may not be recorded such as illegal slaughter (Fakour and Meshgi, 2011; Meshgi and Khodaveisi, 2014).

In Iran, Dicrocoelium has been previously reported based on slaughterhouse inspection at prevalence rates ranging from 1.79% to 0.34% in sheep, 1.47% to 0.69% in cattle and 2.1% to 0.1% in goat in the province of Fars (Ansari-Lari and Moazzeni, 2006). The parasite has been found infecting sheep and cattle in north Khorasan at a prevalence rate of 4.54% and 11.03%, respectively (Oryan et al., 2011). In Guilan province, Dicrocoelium is present at higher prevalence rates in sheep (85%), cattle (66%) and goat (23.25%) (Ahmadi et al., 2010). In the present study, the highest frequency was observed in sheep (36.21%) and cattle (9.37%) from the Guilan province and then found in sheep (21.35%) and cattle (4.16%) from Mazandaran province. In addition, the infection intensity based on the number of Dicrocoelium eggs per gram of feces was higher in sheep samples compared with the cattle. The average of EPG of feces was determined to be 20.81 and 6.86 in sheep and cattle from Guilan province, respectively. Furthermore, the average number of EPG was 6.76 and 2.28 in sheep and cattle from Mazandaran province, respectively. In the Golestan Province, frequency and intensity of infection was found at very low level.

Although these three province areas were close to each other in the littoral states of the Caspian Sea, they have different climates and weather. Rainfall, vegetation, soil type, relative humidity and altitude from the sea are different in the three provinces. Dicrocoelium intermediate host snail has been described to be a land snail which is placed on the ground or on the stem of forage. Nature of interest for the first intermediate host of Dicrocoelium is dry and calcareous or alkaline soils (Manga-Gonzalez et al., 2001). In all three provinces, infection in sheep has always been more than cattle. It is believed that sheep, particularly in Guilan and Mazandaran provinces are responsible for the distribution of infection of Dicrocoelium because of their higher population and free roaming in the environment. The high level of environmental contamination with parasite eggs should be expected daily providence if about 20 Dicrocoelium eggs per gram of faeces is released by sheep (Otranto and Traversa, 2002).

On the other hand, intermediate host snails and ants have a high population in pastures. A Dicrocoelium eggs have created up to 600 cercariae in the snails, which ultimately leads to infection of a lot of ants with metacercaria. Subsequently, an ant can develop up to 300 metacercaria and then eating an infected ant can create a lot of adult worms in the final host, as a result, it should be interpreted that the widespread infection of Dicrocoelium will be achieved (Manga-Gonzalez et al., 2010; Otranto and Traversa, 2002).

In conclusion, the results of the current comprehensive survey showed that Dicrocoelium has been increasing in the three provinces in the littoral states of the Caspi-

an Sea. Guilan and Mazandaran have a high incidence, which emphasizes the need for a comprehensive study, and development of control program in endemic regions of Iran.

Acknowledgments

The authors would like to thank from all people who collaborated in this survey. Project support (No. 31383), was provided by the Center for Research of Endemic Parasites of Iran (CREPI). The authors thank gratefully the CREPI support.

References

Ahmadi, R., Sikejor, E.M., Maleki, M. (2010) Prevalence of *Dicroelium dendriticum* infection in cattle, sheep and goat in Gilan province, Northern Iran. J Anim Vet Adv. 9: 2723-2724.

Ansari-Lari, M., Moazzeni, M. (2006) A retrospective survey of liver fluke disease in livestock based on abattoir data in Shiraz, south of Iran. Prev Vet Med. 73: 93-96.

Beiramvand, M., Akhlaghi, L., Fattahi Massom, S.H., Meamar, A.R., Motevalian, A., Oormazdi, H. (2013) Prevalence of zoonotic intestinal in domestic and stray dogs in rural area of Iran. Prev Vet Med. 109: 162-167.

Ezatpour, B., Hasanvand, A., Azami, M., Mahmoudvand, H., Anbari, Kh. (2014) A slaughterhouse study on prevalence of some helminths of cattle in Lorestan province, west Iran. Asian Pacific J Trop Dis. 4: 416-420.24.

Fakour, Sh. and Meshgi, B. (2011) Determination of antigenic protein of *Dicrocoeliun dendriticum* in naturally infected sheep. J Vet Res. 66: 61-65.

Kose, A., Eser, M., Kartal, K., Bozkurt, M. (2015) Infections of larval stages of *Dicrocoelium dendriticum* and Brachylaima sp. in brown garden snail, *Helix aspersa*, in Turkey. Korean J Parasitol. 53: 647-651.

Manga-Gonzalez, M.Y., Ferreras, M.C., Campo, R., Gonzalez-Lanza, C., Perez, V., Garcia-Marin, J.F. (2004) Hepatic marker enzymes, biochemical parameters and pathological effects in lambs experimentally infected with *Dicrocoelium dendriticum*. Parasitol Res. 93: 344-355.

Manga-Gonzalez, M.Y., Gonzalez-Lanza, C., Cabanas, E., Campo, R. (2001). Contributions to and review of dicrocoeliosis, with special reference to the intermediate hosts of *Dicrocoelium dendriticum*. Parasitology. 123: S91-S114.

Manga-Gonzalez, M.Y., Quiroz-Romero, H., Gonza´lez-Lanza C, Minambres, B., Ochoa, P. (2010) Strategic control of *Dicrocoelium dendriticum* (Digenea) egg excretion by naturally infected sheep. Vet Med. 55: 19-29.

Maurelli, M.P., Rinaldi, L., Capuano, F., Perugini, A.G., Veneziano, V., Cringoli, G. (2007). Characterization of the 28S and the second internal transcribed spacer of ribosomal DNA of *Dicrocoelium dendriticum* and Dicrocoelium hospes. Parasitol Res. 101: 1251-1255.

Meshgi, B., Eslami, A., Taefie-Nasrabadi, N. (2012) Variations of somatic protein profiles of *Dicrocoelium dendriticum* in Iranain ruminants. Iran J Vet Med. 4: 95-98.

Meshgi, B., Khodaveisi, M. (2014) Determination of immunodominant antigens of *Dicrocoelium dendriticum* by hyperimmune sera. Immunol Infect Dis. 2: 4-8.

Ohotori, M., Aoki, M., Itagaki, T. (2014) Distinct distribution of *Dicrocoelium dendriticum* and *D. chinensis* in lwate prefecture, Japan, and a new final host record for *D. chinensis*. J Vet Med Sci. 76: 1415-1417.

Oryan, A., Mansourian, M., Moazeni, M., Nikahva,l B., Barband, S. (2011) Liver distomatosis in cattle, sheep and goats of

northeastern Iran. Global Vet. 6: 241-246.

Otranto, D., Rehbein, S., Weigl, S., Cantacessi, C., Parisi, A., Paolo Lia, R., Olson, P.D. (2007) Morphological and molecular differentiation between *Dicrocoelium dendriticum* (Rudolphi, 1819) and *Dicrocoelium chinensis* (Sudarikov and Ryjikov, 1951) Tand and Tang, 1978 (Platyhelminthes: Digenea). Acta Trop. 104: 91-98.

Otranto, D., Traversa, D. (2002) A review of dicrocoeliosis of ruminants including recent advances in the diagnosis and treatment. Vet Parasitol. 107: 317-335.

Rehbein, S., Lindner, T., Kokott, S. (2002) *Dicrocoelium dendriticum* infection in sheep: faecal egg count, gall bladder egg count, gall bladder fluke count, total fluke count. Helmithology. 2: 71-75.

مجله طب دامی ایران، ۱۳۹۷، دوره ۱۲، شماره ۱، ۳۳-۲۷

میزان شیوع و شدت آلودگی نشخوارکنندگان به دیکروسلیوم دندریتیکم در سه استان ساحلی دریای خزر

مرتضی مجیدی راد[۱] بهنام مشگی[۱*] سعید بکایی[۲]

۱) گروه انگل‌شناسی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران
۲) گروه اپیدمیولوژی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

(دریافت مقاله: ۱۳ تیر ماه ۱۳۹۶، پذیرش نهایی: ۱۰ مهر ماه ۱۳۹۶)

چکیده

زمینه مطالعه: دیکروسلیوم دندریتیکم، ترماتود کوچک کبدی در طیف گسترده‌ای از میزبان‌های اصلی بخصوص نشخوارکنندگان و انسان زندگی می‌کند. ابتلا به این ترماتود دیژن به دلیل حذف مستقیم کبدهای آلوده در بازرسی کشتارگاهی، باعث ضرر و زیان اقتصادی شدیدی می‌گردد. **هدف:** نظر به اهمیت آلودگی در ایران، هدف از بررسی حاضر تعیین میزان شیوع و شدت آلودگی گاو و گوسفند به دیکروسلیوم در سه استان خزری شامل، گیلان، مازندران و گلستان بود. **روش کار:** بدین منظور در هر استان ۴ شهر و در هر شهر ۴ روستا انتخاب شد. در کل ۱۳۴۴ نمونه مدفوع از گاو و گوسفند (۲×۶۷۲) اخذ و آزمایش گردید. در هر نمونه تعداد تخم در گرم مدفوع (E.P.G) براساس روش شناورسازی تعیین شد. **نتایج:** در هر استان شیوع آلودگی به دیکروسلیوم در گوسفند حدود ۴ تا ۵ برابر گاو بود. متوسط آلودگی در استان گیلان برابر با ۳۶/۲۱٪ در گوسفند و ۹/۳۷٪ در گاو بود. میانگین آلودگی در گوسفند و گاو در استان مازندران به ترتیب ۲۱/۳۵٪ و ۴/۱۶٪ و بیشترین میزان شیوع در هر دو میزبان مربوط به چالوس و نور بود. متوسط آلودگی گوسفند و گاو در استان گلستان به ترتیب ۶/۸۷٪ و ۱/۸۷٪ بود. اگر چه میانگین تعداد تخم دیکروسلیوم در هر گرم مدفوع در گوسفندان گیلان، مازندران و گلستان به ترتیب ۲۰/۸۱، ۶/۷۶ و ۲/۴۴ بود ولی در گاو به ترتیب ۶/۸۶، ۲/۲۸ و ۱/۵ گزارش گردید. **نتیجه‌گیری نهایی:** نتایج تحقیق حاضر نشان می‌دهد، نه تنها میزان شیوع آلودگی که شدت آلودگی به دیکروسلیوم همواره در گوسفند بالاتر از گاو است، همچنین بیشترین میزان آلودگی در هر دو میزبان مربوط به استان گیلان است که حدود دو برابر استان مازندران می‌باشد. بنابراین با توجه به شیوع بیشتر آلودگی در دو استان اخیر و اهمیت گوسفند در پراکنش آلودگی ضرورت دارد تدابیر شدید کنترلی با ارائه برنامه‌ای مدون در مناطق مورد نظر صورت پذیرد.

واژه های کلیدی: دیکروسلیوم، شدت، شیوع، نشخوارکنندگان، ترماتود

*) نویسنده مسؤول: تلفن: ۶۱۱۱ (۲۱)۹۸+ نمابر: ۶۶۹۳۳۲۲۲ (۲۱)۹۸+ Email: bmeshgi@ut.ac.ir