

## Original Article

# Morphological Study of *Fasciola* Parasites Isolated from Cattle and Sheep in Golestan Province (Iran)

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## Abstract

**Background:** The genus *Fasciola* parasite causes fascioliasis infection. Fascioliasis is widespread all around the world and it is finding in abundance in the northern provinces of Iran. Cattle and sheep are the main hosts of the *Fasciola* parasite and intermediate hosts are lymnaeid snails such as *Galba* and *Fossaria*. Two main species of this genus are *F. hepatica* and *F. gigantica*. One of the most important methods of diagnosing this worm is morphological method. The aim of this study is to identify *Fasciola* through the morphological method in Golestan province.

**Materials and Methods:** *Fasciola* worms taken from infected livestock livers were washed three times with PBS and were stained with carmine alum. After staining using Valero and Periago methods, the worms were measured morphologically by calibrated microscope, stereomicroscope, and True Chrome II camera. SPSS version 19 was used for analysis of the data.

**Results:** A total of 45 livers from infected sheep and cattle with *Fasciola* worms were taken out of 228 samples, including 84 *Fasciola hepatica* (36.18%), 117 *Fasciola gigantica* (51.31%) and 27 *Fasciola* sp. (11.84%).

**Conclusion:** This study showed that the two main species of worms that is *F.hepatica* and *F. gigantica* were found in abundance in Golestan province. The current study was unable to identify 11.84% genus *Fasciola* showed as *Fasciola* sp.

**Keywords:** Morphological, *Fasciola*, cattle, sheep and Golestan

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## Introduction

Fascioliasis one of the important diseases in animals and humans caused by genus *Fasciola*, two pathogenic species of this genus are *F. hepatica* and *F. gigantica*<sup>1</sup>. This illness has traditionally been considered as a veterinary problem that causes important economic losses thanks to its impact on

livestock, especially sheep and cattle<sup>2</sup> and of only secondary impact on humans<sup>3</sup>.

Cattle and sheep are the main hosts of the *Fasciola* parasite and intermediate hosts are lymnaeid snails such as *Galba* and *Fossaria*<sup>4</sup>. This parasite is widespread around the world and in Iran *F. hepatica* and *F. gigantica* coexist<sup>5,6</sup>. One of the most common methods for detection genus *Fasciola* in the 1970s was

based on morphological properties of the parasite such as fluke length, fluke breadth, cephalic cone length, cephalic cone breadth, testes length<sup>7</sup>.

In recent years, several studies were done by morphological method<sup>5,8,9,10</sup>. In the past two decades, two large outbreaks of human fascioliasis occurred in Guilan province in northern Iran<sup>6,11</sup> as well as one epidemic human fascioliasis was reported in the western province of Kermanshah in Iran<sup>12,13,14</sup>. Several studies about fasciola parasite carried out in Guilan, Kermanshah and some other provinces in Iran<sup>12,15</sup> but, few if any studies have been done in this field in Golestan province. To this end, the aim of this study was to do a morphological scrutiny and species identification of *Fasciola* parasites isolated from cattle and sheep in Golestan province.

## Methods

Liver flukes were obtained from infected cattle and sheep in several slaughterhouses of Gorgan, Gonbad Kavus, Ramian, Azad shaher, Aliabad, Minoodasht, Aqqala, Bander Turkman and Kalaleh in Golestan province, in northeast of Iran. Figure 1 shows Golestan province and the locations where the samples were collected. Liver flukes collection was carried out daily in the region during a one year period (December 2014 to December 2015). The liver worms were carefully separated and fixed in formalin between a slide and cover glass then they were stained with carmine alum and mounted entellan<sup>16</sup> and measured morphometrically by calibrated microscope, stereomicroscope, and True Chrome II camera, which was installed on them. All standardized measurements of flukes measured according to methods proposal by Valero and colleagues<sup>8,17,18</sup>.

Recently, researchers have used the technique to identify morphometric fasciolids<sup>5,8,19</sup>. Parameter measurements including: Body length (BL), Body width (BW), Cone length (CL), Cone width (CW), Oral sucker maximum diameter (OS<sub>max</sub>), Oral sucker minimum diameter (OS<sub>min</sub>), Ventral sucker maximum diameter (VS<sub>max</sub>), Ventral sucker minimum diameter (VS<sub>min</sub>), Distance between anterior end of body and VS (A-VS), Distance between suckers (OS-VS), Pharynx length (PhL), Pharynx width (PhW)<sup>5,8,19</sup>.

SPSS (Statistical Package for Social Sciences) for windows version 19 was used for the analysis of the data. ANOVA test was used to determine the significance of difference among the groups, and t test was used to compare the two hosts in cattle and sheep (Table 1).

## Results

Figures 2 and 3 demonstrate the measurements carried out with calibrated microscope (Leica Galen III) and stereomicroscope (Optica). The results of ANOVA showed that among factors measured there were significant differences among body length, body width, BL/BW ratio, Cone length, VS min and VS max while the following factors Cone width, A-VS, OS-VS, Ph L, Ph W, OS max and OS min were not significantly different ( $P \leq 0.05$ ) (table 1). T test results showed a significant difference in the size of the body length, body width, BL/BW ratio, Cone length, Pharynx length, VS max and VS min worms isolated from cattle and sheep ( $P \leq 0.05$ ).

In the current study, out of 31 infected livers of sheep and 14 infected livers of cattle, 228 flukes of *Fasciola* were collected altogether. With regard to variety of infection, five worms were collected from each infected liver on average. Based on morphological criteria in the method, 84 (36.84%) worms were identified as *Fasciola hepatica*, 117 (51.31%) as *Fasciola gigantica* and 27 (11.84) as *Fasciola sp.* The findings of this research were according to different towns and *Fasciola* species listed in Table 2. In all towns under investigation, two species of *Fasciola* were identified and isolated. In two locations that is, Aq Qala and Minoodasht *Fasciola gigantica* and *Fasciola hepatica* were not identified respectively. In the study out of 31 infected livers of sheep 145 *Fasciola* worms were isolated from which 82 species were *F. hepatica* (56.55%), 39 species *F. gigantica* (26.89%) and 24 species were not identified correctly. Besides, out of 14 infected livers of cattle 83 *Fasciola* worms were isolated from which 78 species were *F. gigantica* (93.97%) and two species of *F. hepatica* (2.4%) and 3 species were not identified correctly (Table 3).

**Table 1:** Comparative morphological data of liver flukes for Sheeps and Cattle from Golestan Province in Iran. A-VS: Distance between anterior end of body and VS.

<i>Fasciola</i> measurements (mm)	<i>F. hepatica</i> in Sheep N=82	<i>F. hepatica</i> in Cattle N=2	<i>F. gigantica</i> in Sheep N=34	<i>F. gigantica</i> in Cattle N=83	<i>Fasciola.sp</i> in Sheep N=24	<i>Fasciola.sp</i> in Cattle N=3	P Value
Body length, BL	16.3 – 36.1 26.13 ± 4.24	15.3 – 19.9 17.6 ± 3.25	16.2 – 39.2 30.82 ± 5.06	24.9 – 50.99 34.28 ± 7.19	16.1 – 34.2 25.41 ± 4.91	26.8 – 32.1 29.8 ± 2.73	.000
Body width, BW	7.8 – 17.4 12.26 ± 2.42	7.1 – 8.1 7.6 ± 0.70	5.2 – 12.9 8.27 ± 1.93	4.22 – 10.1 7.03 ± 1.21	6.4 – 13.8 9.60 ± 2.08	5.9 – 8.1 7.06 ± 1.1	.000
BL/BW ratio	1.61 – 3.50 2.19 ± 0.44	1.8 – 2.8 2.3 ± 0.70	2.67 – 6.32 3.83 ± 0.84	2.64 – 9.76 5.08 ± 1.63	2.09 – 3.91 2.68 ± 0.44	3.96 – 4.54 4.25 ± 0.29	.000
Cone length, CL	1.46 – 3.38 2.31 ± 0.48	2.15 – 2.19 2.17 ± 0.02	1.41 – 3.31 2.54 ± 0.42	1.65 – 4.45 2.9 ± 0.63	1.73 – 3.11 2.28 ± 0.41	2.55 – 3.28 2.85 ± 0.38	.000
Cone width, CW	2.31 – 5.23 3.70 ± 0.77	2.99 – 3.02 3 ± 0.02	1.92 – 4.62 3.47 ± 0.61	1.4 – 4.41 3.48 ± 0.51	1.90 – 4.39 3.38 ± 0.67	3.81 – 3.96 3.87 ± 0.07	.284
A-VS	1.52 – 3.66 2.43 ± 0.49	1.97 – 2.27 2.12 ± 0.21	1.58 – 3.21 2.21 ± 0.48	1.47 – 3.77 2.33 ± 0.46	1.07 – 3.19 2.21 ± 0.44	2.41 – 2.68 2.51 ± 0.14	.244
Distance between suckers (OS-VS)	0.99 – 2.88 1.79 ± 0.46	1.48 – 1.85 1.66 ± 0.26	0.6 – 2.44 1.53 ± 0.4	0.9 – 2.96 1.71 ± 0.43	0.73 – 2.55 1.59 ± 0.39	1.62 – 2.12 1.85 ± 0.25	.192
Pharynx length, PhL	0.31 – 1.03 0.67 ± 0.15	0.35 – 0.37 0.36 ± 0.01	0.37 – 0.98 0.64 ± 0.15	0.32 – 1.15 0.6 ± 0.13	0.49 – 0.99 0.63 ± 0.17	0.59 – 0.65 0.62 ± 0.03	.293
Pharynx width, PhW	0.27 – 0.88 0.44 ± 0.12	0.22 – 0.31 0.26 ± 0.06	0.29 – 0.83 0.48 ± 0.12	0.25 – 0.73 0.45 ± 0.09	0.21 – 0.77 0.41 ± 0.12	0.41 – 0.49 0.46 ± 0.04	.093
Oral sucker maximum diameter (OS max)	0.51 – 1.22 0.82 ± 0.16	0.78 – 0.84 0.81 ± 0.04	0.66 – 1.16 0.87 ± 0.13	0.55 – 1.06 0.83 ± 0.12	0.44 – 1.2 0.83 ± 0.2	0.84 – 0.99 0.9 ± 0.07	.416
Oral sucker minimum diameter (OS min)	0.34 – 0.99 0.62 ± 0.12	0.38 – 0.64 0.51 ± 0.18	0.49 – 0.96 0.67 ± 0.13	0.41 – 0.98 0.63 ± 0.13	0.36 – 0.88 0.57 ± 0.13	0.56 – 0.82 0.65 ± 0.14	.111
Ventral sucker maximum diameter (VSmax)	0.88 – 3.31 1.34 ± 0.33	1.26 – 1.40 1.33 ± 0.09	1.26 – 2.36 1.70 ± 0.23	1.14 – 2.96 1.81 ± 0.29	0.77 – 2.11 1.49 ± 0.36	1.22 – 1.84 1.61 ± 0.34	.000
Ventral sucker minimum diameter (VSmin)	0.81 – 2.31 1.22 ± 0.24	1.24 – 1.29 1.26 ± 0.03	0.96 – 2.15 1.56 ± 0.22	0.82 – 2.54 1.66 ± 0.26	0.63 – 2.06 1.35 ± 0.37	1.06 – 1.77 1.49 ± 0.38	.000

## Discussion

The main goal of the current research was to identify *Fasciola* species by morphological methods. Various methods are used to determine the species of *Fasciola*<sup>4,20</sup>. One of the most important methods for detection *Fasciola* is based on morphological differences in species<sup>5,10,18</sup>. Valero (2001) used *Fasciola hepatica* found in Altiplano in Bolivia as a

standard representatives of this species and Bargues (2005) used samples from Burkina Faso as standard representatives of *F. gigantica* because *Radix natalensis* is the only lymnaeid species (no *Galba/Fossaria*) in that country<sup>21,22</sup>. In the past two decades researchers modified morphological methods were used to identify the species *Fasciola* and this modified method called computer image analysis system (CIAS)<sup>5,8,9</sup>. In the current study, CIAS methods

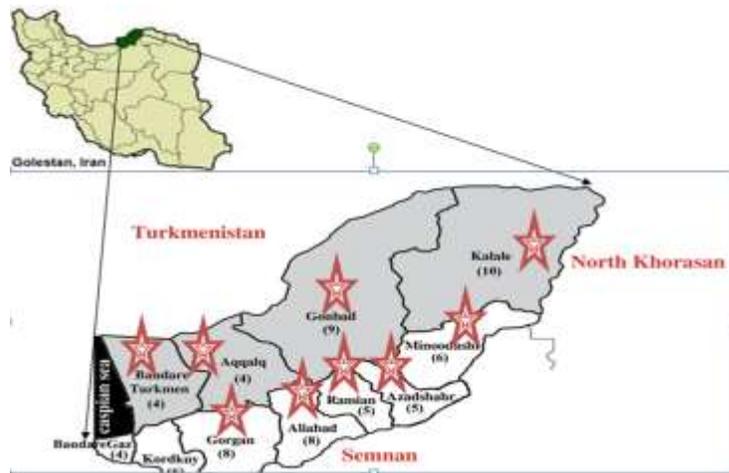


Figure 1. It shows Golestan province and locations where the samples were collected.

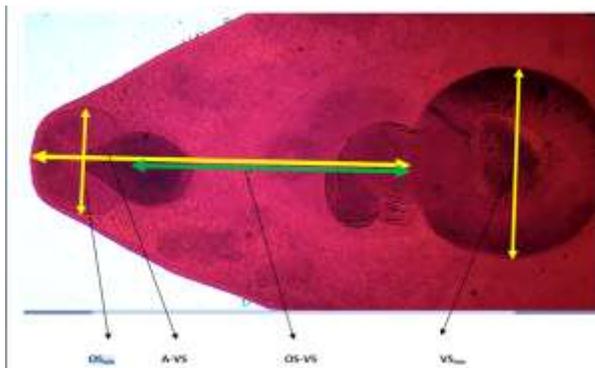


Figure 2. It shows photo taken with a calibrated microscope (Leica Galen III).

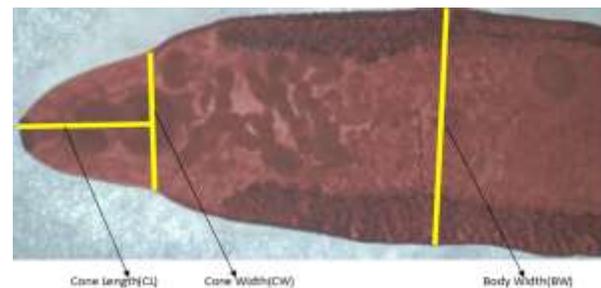


Figure 3. It shows photo taken with a calibrated stereomicroscope (Optica).

were used for detection of *Fasciola* species. Periago et al (2006) using (CIAS) succeeded in identifying *F. gigantica* from *F.hepatica* in both Europe and Africa<sup>23</sup>. The results of this research are shown in Table 1. Most species isolated from Golestan province's was *Fasciola gigantica* (51.31%) Table 1 shows the measured factors of *Fasciola* worms in Golestan province, the analysis of which was performed using SPSS software. According to the table, the average body length of *F. hepatica* worms in cattle is 17.6 mm but in sheep 26.1 mm. In addition, the average body length of *F. gigantica* in cow is 34.28 mm but in sheep 30.11 mm.

There are significant differences in the body length of parasites. The average Body width of *F. hepatica* worms in cattle is 7.6 mm but in sheep 12.2 mm. Ashrafi et al. (2015) conducted a study in the province of Guilan and found out that the dominant species on the plains and low altitudes was

*F.gigantica* and at higher altitudes, the species was *F. hepatica*<sup>24</sup>. Given that towns in the Golestan province were in the low-lying plains, *F. gigantica* isolated in the region is more consistent with the Ashrafi's study. It was also indicated that most sheep in Golestan were infected with *F. hepatica*. On the other hand, most cattle were infected with *F. gigantica* that is in line with Rokni et al.'s study (2010)<sup>25</sup>. The findings of the present study showed that 36.84% from *Fasciola* was of *F. hepatica* species. Therefore, two species of *Fasciola* were found in Golestan province. Periago et al. (2006) believe that if two species of *F. hepatica* and *F. gigantica* coexist in an area, the intermediate form will be reported as *Fasciola sp.* Based on this study and according to Periago et al.'s research report 27 out of 228 samples investigated in the current study were not identified and were equivalent to 11.84% of all sample worms indicated as *Fasciola sp.* In this study, 88.16% of worms were identified by morphological methods. Although this method is time-

**Table 2:** List of towns under investigation and separated species of *Fasciola*.

City	Species		<i>F. hepatica</i>		<i>F. gigantica</i>		<i>Fasciola.sp</i>		Total	
Gorgan	5	10.2%	33	67.3%	11	22.4%	49	100%		
Gonbad-e kavus	5	14.28%	27	77.1%	3	8.57%	35	100%		
Aliabad	18	54.54%	12	36.36%	3	9.09%	33	100%		
Ramian	10	50%	10	50%	-	-	20	100%		
Azadshaher	13	52%	9	36%	3	12%	25	100%		
Bandar turkman	17	68%	4	16%	4	16%	25	100%		
Aq Qala	14	87.5%	-	-	2	12.5%	16	100%		
Minudesht	-	-	18	100%	-	-	18	100%		
Kalaleh	2	28.57%	4	57.14%	1	14.28%	7	100%		
Total	84		117		27		228			

**Table 3:** Species identified based on host in Golestan province.

Total	Cattle			Total	Sheep		
	<i>Fasciola sp</i>	<i>F. hepatica</i>	<i>F. hepatica</i>		<i>Fasciola sp</i>	<i>F. gigantica</i>	<i>F. hepatica</i>
83	3	78	2	145	14	39	82
100%	3.61%	93.97%	2.4%	100%	16.55%	26.89%	56.55%

consuming and cumbersome in nature, morphological identification method is an appropriate way to identify *Fasciola gigantica* from *Fasciola hepatica*.

## Conclusion

This study indicated that the main two species of worms, that is *F. hepatica* and *F. gigantica* were found in abundance in Golestan province. These two species of *Fasciola* in the area under investigation were found in overlapping coexistence. The current study was unable to identify 11.84% genus *Fasciola* showed as *Fasciola sp*.

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