Morphologic and morphometric variations of the adult and the eggs of frequent Fasciola species from domestic ruminants of North West of Iran

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Abstract

Worldwide including Iran, Fasciola species are the causes of human and animal fasciolosis which have comparatively identical morphologies. The present study is aimed to identify different Fasciola forms by using morphologic and morphometric variations from domestic ruminants of northwestern Iran. A total of 130, 67 and 140 livers of cattle, water buffaloes and sheep respectively were collected from Urmia slaughterhouse. The adult helminths were removed and stained using Acetocarmine staining. The Fasciola eggs were directly extracted from the uterus of adult helminths. The overall frequency of infection was 28.19%. The highest infection rate was found in water buffalos (34%). The highest number of helminth per each animal was recorded for cattle (9.23%). The predominant infecting fasciolid in the examined ruminants was F. gigantica (51.89%) from water buffaloes origin. There was a significant difference among the width (W), the length (L), and the distance between ventral sucker to the posterior end of the body (DBVE) of all Fasciola forms from the examined animals. The L of different Fasciola forms had a significant difference for F. gigantica and intermediate form of Fasciola from cattle and water buffaloes origins. The W and the proportion of the body length to the width (SI) of eggs from all Fasciola forms had no significant difference. The Ls of eggs of F. hepatica and F. gigantica were significantly different. The results of this study elucidated three forms of Fasciola co-existing in the ruminants of the region. Additionally, the morphology and morphometry of adult and eggs of Fasciola species within a range of hosts may be taxonomically informative and one of the character sets for discrimination of fasciolid forms.

Key words: Fasciola; Egg; Ruminant, Iran

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Introduction

Fasciolosis is a helminth born parasite disease worldwide which is remarkable in the region (Imani-Baran et al., 2012). The disease is an old parasitic infection (Kendall and McCullough, 1951). In Europe, it was believed that the presence of infection dates back to the Mesolithic and Neolithic periods, namely 5000-5100 years ago (Mas-Coma, 2003). Classically, the prevalence of fasciolosis has a "Spotty distribution" style. However, in spite of the old age of the disease and considering the economic damages which it imposes on animal husbandry industry directly or indirectly, around 2.4 million people in the world are affected by this parasite and about 80 million people are at risk of infection (Massoud, 1974). In terms of Veterinary, many domesticated herbivorous animals become affected by metabolic and liver disorders due to being infected with the parasite, and they will not have any economic efficiency (Ghobadi and Yakhchali, 2003, Yakhchali and Ghobadi, 2005, Eslami, 2008).

Fasciola is a prevalent trematode of bile ducts which is reported from humans and a range of animals, i.e. cattle, buffalo, sheep, goat, donkey, horse, rabbit, pig (Rim et al., 1994, Salahi-Moghaddam, 2004). The presence of the intermediate form of Fasciola in east Mediterranean countries was an issue (Farag et al., 1979). In Asia, especially in Japan, Taiwan, Philippines and South Korea, morphologically, Fasciola species showed remarkable variations (Watanabe, 1962, Oshima et al., 1968, Akahane et al., 1970, Kimura et al., 1984, Srimuzipo et al., 2000). In the Middle East, the intermediate form of Fasciola was reported from the Gilan Province in the north of Iran. Sahba et al. (1972) reported the first three Fasciola species as F. hepatica, F. gigantica, and F. indica by using morphometric analysis. Since Fasciola species are different in transmission, epidemiology, phylogenic characteristics and intermediate host of lymnaeid snails, differentiation of Fasciola species is of great importance in prevention of the outbreak of fasciolosis (Massoud, 1974, Mas-Coma and Bargues, 1997, Bargues et al., 2002). The morphologic characteristics of adult and eggs of Fasciola are influenced by the age of the helminth, host, and method of preparing the specimens (Ashrafi et al., 2006). Cross-fecundation of both F. hepatica and F. gigantica produces hybrid type, which causes morphological mistakes with other species (Agatsuma et al., 2001).

In Asia, especially in Iran, the prevalence of Fasciola species has an overlap. F. hepatica is mostly prevalent in the high and moderate areas, while F. gigantica exists in low land and tropical areas of the country (Agatsuma et al., 2001). Furthermore, human fasciolosis is ecologically common in the areas with F. hepatica and F. gigantica overlap (Cheng and Bogitsh, 1998). Thus, the present study was performed to compare morphologic and morphometric characteristics of adult helminths and eggs of Fasciola species from domestic ruminants of northwestern Iran.

Materials and methods

Collection of fasciolid helminths

The livers of the ruminants (130 breeds of cattle, 67 buffaloes and 140 sheep) were collected from Urmia industrial slaughterhouse, and were transferred to Parasitology laboratory. The inflected livers were dissected and the helminths were extracted. They were washed several times in 0.01M phosphate buffer saline (PBS, pH=7.2), were stained using Asetokarmin, and were examined under light microscope at 100× magnification. Fasciola species were identified using key identification described by Soulsby (1982) and Moghaddam et al. (2004). The Fasciola eggs were directly extracted from the uterus of adult helminths by using dissecting needle and were washed within 0.01M PBS (pH=7.2). The eggs were incubated at +4°C until examination.

Fasciolid forms and eggs examination
A total number of 300 Fasciola species, i.e. 150 F. hepatica from cattle and 150 F. gigantica from water buffaloes were examined using optic microscopy and then were photographed. The all examined Fasciola species were also compared with donated specimens of F. hepatica and F. gigantica which were molecularly standard by Yakhchali et al. (2015) (Table1). To determine the size of fasciolid helminths, the distance between the suckers (DBS), the distance between the ventral sucker and the posterior end of body (DBVE), body length (L), body width (W), and proportion of body length to width (SI) were considered. To find out egg morphology and morphometry, a total number of 100 Fasciola eggs from cattle and sheep were individually measured and recorded using an Olympus compound microscope with an ocular micrometer at 1000×.

### Table 1. The prevalence of Fasciola species from the examined ruminants of northwestern Iran (n=337).

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of examined animals</th>
<th>Prevalence (n/N, %)</th>
<th>No/H (%)</th>
<th>Fasciola species</th>
<th>Fh</th>
<th>Fg</th>
<th>InF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>120</td>
<td>30.7</td>
<td>9.23</td>
<td>8.94</td>
<td>23.99</td>
<td>17.93</td>
<td></td>
</tr>
<tr>
<td>Water buffaloes</td>
<td>67</td>
<td>34</td>
<td>6.85</td>
<td>4.91</td>
<td>9.96</td>
<td>4.58</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>140</td>
<td>22.8</td>
<td>5</td>
<td>2.97</td>
<td>17.93</td>
<td>8.77</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>337</td>
<td>28.19</td>
<td>7</td>
<td>16.83</td>
<td>51.89</td>
<td>31.28</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Fh, Fasciola hepatica; Fg, Fasciola gigantica; InF, Intermediate form of Fasciola; No/H, number of helminths per each animal.

### Statistical analysis

The body size and measurements of eggs were analyzed by SPSS statistical program (version 14, SPSS Inc., Chicago, Illinois, USA) using two sample t-tests and One-Way ANOVA. A probability of ≤ 0.05 was regarded as significant.

### Results

The overall prevalence was 28.19% with the highest infection rate in water buffaloes (34%). Fasciola gigantica (51.89%) was found to be a prevalent specie in the examined ruminants. The highest number of Fasciola per each animal was recorded for cattle (9.23%) with diversity of F. hepatica (8.94%), F. gigantica (23.99%), and intermediate form (17.93%).

### Morphologic and morphometric variations of adult flukes

F. hepatica was leaf-shaped with an oblique body angle, an evident shoulder and a cephalic cone and an oral sucker with a short distance from ventral sucker in the upper one-third of the body. In F. gigantica and intermediate form, cephalic cone was evident without a shoulder. The number of caecum branches particularly in the internal edge was more than the F. hepatica. The ovary and uterus branches in F. gigantica were also longer than that of F. hepatica.

The micrometry findings of adult fluke are presented in Table 2. The L, W, DBVE, and SI of all three adult Fasciola from all of the examined ruminants had significant differences with exceptions for SI and DBS of F. gigantica from water buffaloes origin (p=0.0001) and DBS of intermediate form of Fasciola from all of the examined animals. The W of intermediate form of Fasciola from water buffaloes origin had a significant difference with cattle and sheep origins. The L had also a significant difference between F. gigantica and intermediate form from cattle and water buffaloes origins (p<0.05).
The eggs’ morphology and morphometry variations

The eggs of Fasciola species were yellow light brown and oval with operculum in the end point. There was undistinguished cellular mass surrounded by a great number of yellow seeds inside the eggs. The seeds were in the middle line of the eggs near to operculum.

The results of micrometry of the eggs from all Fasciola species were shown in Table 3. There was a significant difference between L, W and SI of eggs of F. hepatica from sheep and cattle (p<0.05). The L, W and SI of eggs from all three species of Fasciola had no significant differences (p>0.05). The L and SI of eggs of F. hepatica and the intermediate form of Fasciola from sheep and goats had significant differences (p<0.05).

Discussion

Fasciola species are remarkable liver flukes of ruminants in all areas of Iran which yearly impose direct and indirect economic losses (Moghaddam et al., 2004). In the present study, water buffaloes had the highest prevalence. However, the highest number of helminths per each animal was recorded for the cattle. F. gigantica was found to be a prevalent specie in the examined ruminants in northwestern Iran. The infections with both F. hepatica and F. gigantica have been reported from ruminants of different parts of Iran (Salahi-Moghaddam, 2004, Yakhchali and Ghibadi, 2005, Ashrafi et al., 2006).

The morphologic findings of Fasciola species from the examined ruminants were nearly identical to the reports from other parts of Iran and the world. In Asia, F. hepatica and F. gigantica have overlaps in different areas, i.e. Iran, Japan, Pakistan, Korea, Taiwan and the Philippines (Mas-Coma and Bargues, 1997). All three forms of Fasciola have been reported in Egypt and Japan (Terasaki et al.,

Table 2. The body features of identified Fasciola species from the examined ruminants of northwestern Iran (Mean±SD).

<table>
<thead>
<tr>
<th>Animals</th>
<th>Fasciola species</th>
<th>L (cm)</th>
<th>W (cm)</th>
<th>DBVE (cm)</th>
<th>DBDV (cm)</th>
<th>SI (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Fh</td>
<td>0.24±2.65</td>
<td>0.12±1.36</td>
<td>0.25±2.34</td>
<td>0.06±0.26</td>
<td>0.13±1.93</td>
</tr>
<tr>
<td></td>
<td>Fg</td>
<td>0.29±2.90</td>
<td>0.12±0.78</td>
<td>0.31±2.59</td>
<td>0.06±0.26</td>
<td>3.73±0.53*</td>
</tr>
<tr>
<td></td>
<td>InF</td>
<td>* 0.20±2.52</td>
<td>0.11±1.01</td>
<td>* 0.22±2.254</td>
<td>0.06±0.26</td>
<td>2.5±0.23*</td>
</tr>
<tr>
<td>Water buffaloes</td>
<td>Fh</td>
<td>0.30±1.62</td>
<td>0.14±0.87</td>
<td>0.28±1.36</td>
<td>0.05±0.23</td>
<td>1.85±0.16*</td>
</tr>
<tr>
<td></td>
<td>Fg</td>
<td>0.45±2.87</td>
<td>0.11±0.78</td>
<td>* 0.46±2.62</td>
<td>0.04±0.24</td>
<td>3.64±0.32*</td>
</tr>
<tr>
<td></td>
<td>InF</td>
<td>0.30±1.72</td>
<td>0.17±0.69</td>
<td>1.44±0.29</td>
<td>0.04±0.25</td>
<td>2.42±0.21*</td>
</tr>
<tr>
<td>Sheep</td>
<td>Fh</td>
<td>0.19±2.15</td>
<td>0.41±3.36</td>
<td>* 0.21±1.89</td>
<td>0.07±0.24</td>
<td>1.9±0.16*</td>
</tr>
<tr>
<td></td>
<td>Fg</td>
<td>0.97±0.91</td>
<td>3.07±0.42</td>
<td>* 0.04±0.25</td>
<td>3.66±0.35*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>InF</td>
<td>* 0.10±1.037</td>
<td>2.48±0.27</td>
<td>* 0.04±0.24</td>
<td>2.66±0.19*</td>
<td></td>
</tr>
</tbody>
</table>

Notes: DBDV, distance between dorsal and ventral sucker; DBVE, the distance between ventral sucker and the posterior end of the body; L, body length; SI, proportion of body length to width. (*Significant p<0.05)

Table 3. The features of identified Fasciola species eggs from the examined ruminants of northwestern Iran (Mean±SD).

<table>
<thead>
<tr>
<th>Animals</th>
<th>Fasciola species</th>
<th>L (cm)</th>
<th>W (cm)</th>
<th>SI (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Fh</td>
<td>15.16±242.3</td>
<td>14.43±135.37</td>
<td>0.23±1.78</td>
</tr>
<tr>
<td></td>
<td>Fg</td>
<td>15.32±229.93</td>
<td>13.53±130.77</td>
<td>0.14±1.76</td>
</tr>
<tr>
<td></td>
<td>InF</td>
<td>14.6±234.53</td>
<td>11.88±134.94</td>
<td>0.16±1.73</td>
</tr>
<tr>
<td>Sheep</td>
<td>Fh</td>
<td>233.88±19.92*</td>
<td>140.84±16.09</td>
<td>1.65±0.16</td>
</tr>
<tr>
<td></td>
<td>Fg</td>
<td>16.29±230.73</td>
<td>12.76±138.16</td>
<td>0.15±1.67</td>
</tr>
<tr>
<td></td>
<td>InF</td>
<td>17.87±236.73</td>
<td>16.59±143.58</td>
<td>0.17±1.62</td>
</tr>
</tbody>
</table>
1982, Esteban et al., 1998). Regardless of diploid, triploid and/or mixoploid forms of Fasciola, Agastuma et al. (1994) noted Fasciola reproduction because of abnormal spermatogenesis through parthenogenesis led to the formation of three different genotypes including genotype I (F. hepatica-like), genotype II (F. gigantica-like), and genotype III (intermediate form). Genetically, genotype I was absolutely distinctive from genotype II and genotype III. In different parts of Iran, particularly, in low lands and mountainsides the overlap of Fasciola species and the coinfection infection of the liver were reported (Ashrafi et al., 2006). Recently, an intermediate form of Fasciola has been reported from ruminants of Fars Province in south and Mazandaran Province in the north of Iran (Salahi-Moghaddam, 2004, Karimi, 2008).

The morphometric evaluation of helminths revealed differences among Fasciola species from the examined ruminants. The L of F. hepatica from water buffaloes origin was longer than other Fasciola species from cattle and sheep while, the L of F. hepatica and the intermediate form of Fasciola from water buffaloes origin was respectively longer than the specimens from sheep and cattle origins. Micrometric or even morphological evaluation of body features of Fasciola species may be influenced by final host, immunity of host, intermediate host or various genealogy factors (Dalton, 1999). Sahba (1972) noted that the L of F. gigantica (4.39-5.2 cm) was longer than F. hepatica (1.88-2.33 cm) and another fasciolid with size L in between them was reported as F. indica. Mayne (2000) also reported that the average body size of F. hepatica (1-2x2-3 cm) was less than that of F. gigantica (0.5-1.3x2-4.76 cm).

The SI of F. gigantica and the intermediate form from the examined animals was more than F. hepatica. This finding was in agreement with Malek (1981) and Valero et al. (2001). According to morphometric findings of Fasciola species from the ruminants of northern Iran, the SI for F. hepatica, F. gigantica and intermediate form from sheep origin was 1.90, 4.20, 2.61, and for cattle and water buffaloes the origins were 1.96, 3.46, and 2.2 respectively. In a genomic study by Agatsuma et al. (2000), it was shown that there was cross-hybridization between both F. hepatica and F. gigantica. Ashrafi et al. (2006) reported that the intermediate form of Fasciola from Iran was originally different from the other reports elsewhere. Bargues et al. (2002) confirmed the existence of intermediate fasciolid form of F. hepatica and F. gigantica in Iran. In a descriptive study considering the features of the cephalic cone, L, W, SI, area (A), and perimeter (P) on typical and atypical fasciolids using Computerized Image Analysis System (CIAS), Salahi-Moghaddam (2004) reported that they were morphologically the intermediate forms of Fasciola. According to Ashrafi et al. (2006) the intermediate form of Fasciola was different from other reports. Salahi-Moghaddam (2004) studied atypical helminths beside the typical fasciolids descriptively and confirmed the intermediate form with regard to characteristics such as cephalic cone and SI. Ashrafi et al. (2006) elucidated that F. hepatica was larger than the classic form of F. hepatica. However, F. gigantica was slightly narrower with lesser A and larger sizes than the standard forms from Burkina Faso. The two Fasciola species, i.e. F. california and F. halli were reported according to evolutionary features, geographical distribution and tegument shape from American ruminants (Sinitsin, 1993). In addition, Ali (1993) morphologically reported intermediate forms of F. hepatica and F. gigantica from Egypt.

The morphology of Fasciola species eggs in the present study was in agreement with earlier reports (Soulsby, 1982, Semidt and Roberts, 2000, Eslami, 2008). The eggs of F. hepatica were ovoid, bile-stained and operculated. The L, W and SI of measured eggs of Fasciola species were nearly similar. There was a significant difference between the length of F. hepatica and F. gigantica eggs. The average egg sizes of F. hepatica and F.
gigantica from Iranian ruminants were 60-92 by 128-152µm and 68-94 by 135-190µm, respectively (Salahi-Moghaddam, 2004). In previous reports, the length of eggs of F. hepatica was reported to be less than eggs of F. gigantica (Soulsby, 1982, Mayne, 2000). According to Schmidt and Roberts (2000), the measure of F. hepatica eggs was 63-90×130-150µm in size while F. gigantica eggs was larger and was measured to be 70-90 by 160-190µm. The size of F. gigantica eggs was 90-104 by 156-197µm and the F. hepatica eggs were of size 63-90 by 130-150µm (Soulsby, 1982). Paniker (1997) also reported egg size of F. hepatica was 63-90 by 130-150µm. Parija (1996) also reported egg size of F. hepatica was 63-90 by 130-150µm.

From the results of the present study, the prevalence of Fasciola was indicative in examined ruminants of northwestern Iran. In addition, cattle play an important role as a source of infection for other definitive hosts to which different Fasciola forms co-exist in the ruminants of the region. The morphology and micrometry findings may also be taxonomically informative and reliable to discriminate morphologically and morphpometericaly Fasciola species from different animal origins. These findings were of great importance to humans and ruminants because of the differences in transmission and epidemiology characteristics. Thus, further studies were recommended to be carried out in order to determine the polymorphism of Fasciola species from lymnaeid snails and final hosts.

References
Agatsuma, T., Terasaki, K., Yang, L., Blair, D. (1994) Genetic variation in triploids of Japanese Fasciola species and relationships with other species in the

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مطالعه ریخت شناسی و مورفومتریک تخم و گرم بالغ گونه های فاسیولا انگل کید نشخوار کندگان اهلی شمال غرب ایران

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چکیده
گونه های فاسیولا عامل فاسیولوژیس آنان و دام در ایران و جهان می باشند که تا حدودی ریخت شناسی مشابهی دارند. مطالعه حاضر به منظور شناسایی و مقایسه ویژگی های مورفولوژیک و مورفومتریک گونه های فاسیولا انگل نشخوار کندگان اهلی در شمال غرب ایران انجام شد. تعداد 130، 67 و 140 عدد کبد به ترتیب از گاو، گاومیش و کوسمید جمع آوری شدند و از برخی داند کیف کرم بالغ فاسیولا جمع آوری و رنگ آمیزی استوارنیشند. در نتیجه کلامی که از گاو در 19/28، بیشترین کلامی در گاو و گاومیش (39 درصد) بیشترین نسبت تعداد کرم بالغ فاسیولا به راس دام در گاو (23/9) بود از نظر تعداد فاسیولا رخک (81/5 درصد) گونه شاید سه است. از نظر ساختار فاسیولا، عرض و فاصله بانکش کمی با تا دم در اشکال مختلف فاسیولا جدا شده از گاو، گاومیش و فاسیولا اختلاف معنی داری داشته میانگین طول در گونه های فاسیولا انگل گاو و گاومیش در فاسیولا حد واسطه و فاسیولا رخک دارا می باشد از گاو و گاومیش اختلاف معنی داری بود میانگین عرض و نسبت طول به عرض تخم در گونه های مختلف فاسیولا همانند که در جدول نشان داده شد. اختلاف معنی داری در تعداد خاصی از گاومیش و فاسیولا رخک از گاو در نتیجه حساسیت بیشتر در نشخوار کندگان شمال غرب ایران به علایم آن وجود یافته و ریخت شناسی و مورفومتریک تخم و کرم در بالغ گونه های فاسیولا در میزان های مختلف استفاده کرد. نتیجه آنها از نظر تاسکومیک راهنما و میسر می باشد.

واژگان کلیدی: فاسیولا، تخم، نشخوار کندگان، ایران.

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