Evaluation of two Iranian domestic ovine breeds for their pathological findings to gastrointestinal infection of *Haemonchus contortus*

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Abstract The generally warm, moist environmental conditions in the Northwestern Iran are ideal for survival and growth of the egg and larval stages of *Haemonchus contortus* and other gastrointestinal nematodes (GIN) of sheep and goats. A total of 2,421 animals were slaughtered and examined from July 2010 to July 2011 in Urmia abattoir. In case of sheep, 225 out of 2,421 were positive and prevalence of *H. contortus* infestation was 9.3 %. Sex wise prevalence of *H. contortus* in sheep was 33.08 % (76/229) in male and 66.22 % (149/225) in female. The females indicated significantly ($P < 0.05$) higher prevalence (66.22 %) as compared to males (33.08 %). The

highest prevalence was recorded in the spring (April) and the lowest was in summer (July), respectively. On microscopic examination, infiltration of mononuclear cells and eosinophils in gastric glands, periglandular hyperemia and hemorrhage, mucous gland hyperplasia, connective tissue proliferation and necrosis was observed. Also, in mixed abomasal infection with *Haemonchus* and *Ostertagia* species, mucosal hyperplasia and increased mucous glands and sometimes cystic glands were seen. Statistical analysis using SPSS software, and Chi-square test, demonstrated a non-significant difference between ages and abomasal PH values of infected and healthy sheep ($P < 0.05$). But the difference between sexes, seasons and abomasal lesions was significant ($P > 0.05$).

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Introduction

Haemonchus contortus is a blood-feeding gastrointestinal nematode (GIN) that dwells in the abomasum of small ruminants (goats and sheep) particularly in humid temperate and subtropical climates (Amarante et al. 2005). This parasite can cause severe production losses in these animals. The development of multi-drug resistance by *H. contortus* parasites has driven research into alternative control measures, including selection of sheep genetically resistant to GIN infection. Genetically resistant sheep (either through breeding selection or use of local indigenous breeds) are increasingly considered as viable alternatives to increase animal production (Amarante et al. 2009) and to learn novel mechanisms of resistance not described in commercial breeds (Piedrafita et al. 2010).

Importantly, GIN parasites may not adapt to these resistance mechanisms in such selected sheep (Kemper et al. 2009). However, to date the mechanisms underlying the genetic resistance of sheep to GIN infections are largely unknown. *H. contortus* is of primary concern since it is a highly pathogenic blood-feeder helminth that causes anaemia and reduced productivity and can lead to death in infected animals (Burke et al. 2007). In most production systems lambs are moved from barn to pasture directly after weaning, facing helminth infections for the first time (Strittmatter 2003). The objective of this study was to histopathological study of *H. contortus* in Herrick sheep abomasum.

Materials and methods

Study area

The district Urmia of agro-ecological zone was selected for the present study. Climatically, the study region is subtropical and receives an annual rainfall of about 150–350 mm. The temperature is highest in June, before the onset of monsoon season. During summer, the daily maximum temperature exceeds 45 °C and rarely declines below 22 °C. Relative humidity is lowest during April–May and rises during the monsoon season. One year cycle is divided into four seasons viz. winter (December–February) spring (March–April) summer (May–September) and autumn (October–November). Summer also includes monsoon season (July–August)).

Animals and experimental design

During the period from July 2010 to July 2011, a total of 2,421 Herrick sheep ranging from 2 to 5 years of both sexes were subjected to a careful post-mortem examination during abattoir inspection in Urmia slaughterhouses in West Azarbijan province (coordinates: 37°33N, 45°04E) Iran. The Herrick and Afshari sheep were selected to be examined as they are the most important and numerous native breed. The resulting samples were sent to the Laboratory of Parasitology and pathology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, for parasitological and histopathological examination.

Parasitological and histopathological analysis

Abomasa of total 2,421 sheeps slaughtered at Urmia abattoir were examined for the presence of adult worm of *H. contortus*. Sheep were comprising on male ($n = 859$) and female ($n = 1562$). Each abomasum was separated from the small intestine and the abomasums transferred to the parasitology laboratory, and examined within 3 h of

slaughter. The abomasum was slit open and their contents washed gently with water in separate buckets and collected. In addition, the abomasum was divided longitudinally in two equal parts (towards the greater curvature with a pair of scissors) and the whole mucosa of one part was scraped off and digested in a pepsin–HCl solution for 10 h at 37 °C. The digest was concentrated over a 400-mesh sieve (aperture diameter 51 μ m) under a water-jet and the entire sieve contents examined (Mir et al. 2007; Urquhart et al. 1987). A 10 mm-long sample of the abomasa segment without any injury was removed, fixed, and prepared for histopathological examination. Tissue fragments were fixed in 10 % neutral buffered formalin solution (for 72 h), after stabilizing embedded in paraffin, sectioned at 5 μ m thickness and then, stained with hematoxylin and eosin (H&E).

Statistical analysis

Statistical analysis using SPSS software and Chi-square test was applied for the statistical analysis of the data (Petrie and Watson 1999).

Results

Overall prevalence of *Haemonchus contortus* in sheep

In this study, 225 abomasa (9.3 %) were infected with *H. contortus*. Sex wise prevalence of *H. contortus* in sheep was 33.08 % (76/229) in male and 66.22 % (149/225) in female. In the monthly survey, the highest rate of infection was in April (22.9 %) and the lowest was in July (14.9 %). The seasonal survey shows that the highest and lowest infection rates are in the spring (13.6 %) and in summer (2.4 %), respectively. In statistical analysis, there was a significant difference between seasons and the prevalence of the parasite ($P < 0.05$). The study of parasites in the abomasa of 2,421 sheep revealed that acute and chronic forms of *haemonchosis* are common in the region. And preacute form of infection has no sign of presence or is utterly rare in above-mentioned region. (Tables 1, 2; Chart 1).

Macroscopic and microscopic findings

Petechial hemorrhage in the abomasal mucosa (Probably due to the attachment of the parasite) (Fig. 1), extensive mucosal hemorrhage, inflammation, mucous secretions around lesions and paleness of internal organs were seen (Fig. 2). Also abomasal contents were fluidal and partially covered with free blood; the carcasses were pale and have

Table 1 Abomasum monthly infestation with *Haemonchus contortus*

Seasons	Intact	Infested	Total number of inspections
July			
Count	160	0	160
Expected count	145.1	14.9	160.0
Monthly summary (%)	100.0	0	100.0
August			
Count	221	2	223
Expected count		20.8	223.0
Monthly summary (%)	202.2	0.9	100.0
	99.1 %		
September			
Count	231	13	224
Expected count	221.2	22.8	224.0
Monthly summary (%)	94.7	5.3	100.0
October			
Count	186	27	213
Expected count	193.1	19.9	213.0
Monthly summary (%)	87.3	12.7	100.0
November			
Count	138	18	156
Expected count	141.4	14.6	156.0
Monthly summary (%)	88.5	11.5	100.0
December			
Count	158	23	181
Expected count	164.1	16.9	181.0
Monthly summary (%)	87.3	12.7	100.0
January	171	11	182
	165.0	17.0	182.0
	94.0 %	6.0 %	100.0 %
February			
Count	122	10	132
Expected count	119.7	12.3	132.0
Monthly summary (%)	92.4	7.6	100.0
March			
Count	73	6	79
Expected count	71.6	7.4	79.0
Monthly summary (%)	92.4	7.6	100.0
April			
Count	194	51	245
Expected count	222.1	22.9	245.0
Monthly summary (%)	79.2	20.8	100.0
May			
Count	270	58	328
Expected count	297.4	30.6	328.0
Monthly summary (%)	82.3	17.7	100.0
June			278
Count	271	7	
Expected count	252.0	26.0	378.0

Table 1 continued

Seasons	Intact	Infested	Total number of inspections
Monthly summary (%)	97.5	2.5	100.0
Total			
Count	2195	226	2421
Expected count	2195.0	226.0	2421.0
Monthly summary (%)	90.7	9.3	100.0

Table 2 Abomasum seasonal contamination with *Haemonchus contortus*

Seasons	Intact	Infected	Total number of inspections
Spring	735 (86.4 %)	116 (13.6 %)	851 (100 %)
Summer	612 (97.6 %)	15 (2.4 %)	627 (100 %)
Autumn	482 (87.6 %)	68 (12.4 %)	550 (100 %)
Winter	366 (93.1 %)	27 (6.9 %)	393 (100 %)
Total	2195 (90.7 %)	226 (9.3 %)	2421 (100 %)

generalized edema and fluid throughout of the body cavities secondary to hypoproteinemia. Moreover, following findings were witnessed: mononuclear cells infiltration (lymphocytes, monocytes and plasma cells), prominent eosinophilic infiltration in mucous glands and some even penetrated to the sub mucosa (Fig. 3), perivascular hyperemia and lymphocytic infiltration, tissue thickening due to an increase in mucous glands and mucous secretion (Figs. 4, 5), connective tissue proliferation and necrosis (in chronic abomasal inflammation and wound healing) (Fig. 6).

Abomasal pH values and relationship between sex, ages, season and prevalence in sheep

With regard to determination of infected abomasal PH values, slight increase in PH was seen (3.5–4.5), but there wasn't a close correlation between PH values of parasitized and healthy abomasa ($P > 0.05$). Also there wasn't a significant difference between ages of infected sheep ($P > 0.05$). But the difference between sexes, seasons and abomasal lesions was significant ($P > 0.05$). Pertaining to seasons in which amount of rainfalls are considerable, temperature would result in dramatic increase of parasite frequency. Subsequently more sheep are afflicted. Needless to say, lesions would be widely seen in the abomasa either. So, there is a direct relevance between season and prevalence of lesions in the abomasa ($P < 0.05$). And this issue occurs during the spring in the North West region of Iran (West Azerbaijan).

Chart 1 Abomasum monthly infestation with *Haemonchus contortus*

Discussion

The results revealed that the infected animals (sheeps) harbour *H. contortus* infection throughout the year at all the studied sites with varied incidence. It could be inferred from the infection level that the permanent flocks in azerbaijan west Urmia areas had substantial worm burdens of *H. contortus*. The present study demonstrated that prevalence of infection was 9.3 % and the average rate of infection is 49 % in Iran (Eslami and Nabavi 1976). So this rate is much less than average of infection in Iran. This is probably due to cold winters and hot summers that destroy the parasite. According to studies by Eslami and Nabavi (1998) rate of infection in sheep is 49 %, and 40.8 % in goats, 0.8 % in wild sheep, 22 % in cattle, and 12 % in camels. The seasonal survey demonstrated that the highest and lowest infection rates are in the spring (13.6 %) and in summer (2.4 %), successively. Recently O'Sullivan and

Donald (1970) suggested that spring rise was due to a temporary depression of immunological capacity brought about by endocrine changes associated with lactation, which could result in a resumption of development of arrested larvae, an increased rate of establishment of newly ingested larvae and increased fecundity of female worms. And also in the monthly survey, the highest rate of infection was in April (22.9 %) and the lowest was in July (14.9 %). Thus Gibbs (1967) have demonstrated that transplantation of such arrested larvae into worm-free, susceptible ewes in winter did not result in the immediate maturation of the parasites. When maturation occurred, it coincided with the time that spring rise regularly takes place in this area (April), some 10-12 weeks after transplanting the arrested larvae. Moreover, numerous attempts to induce maturation of arrested larvae in sheep during the winter by administration of immunosuppressive drugs have failed (Brunsdon 1966; McKenna 1998; Nayeibzadeh et al.

Fig. 1 Petechial in the mucosa of the abomasum

Fig. 2 Hemorrhage in between the glands and inflammatory cell infiltration

Fig. 3 Infiltration of inflammatory cells in the mucosa of the abomasum

Fig. 4 Infiltration of inflammatory cells in the mucosa of the abomasum

Fig. 5 Increase in mucous glands and hemorrhage in between the glands

Fig. 6 Proliferation of connective tissue

2005). In the study, there was an increased number of circulating blood eosinophils in the sheep infected with *Haemonchus* larvae. This was in agreement with Terefe et al. (2005). There is also some evidence suggesting that eosinophils may contribute to pathogenesis during parasitic infection Nickdel et al. (2001). Moreover, it has previously been shown that a number of ovine parasitic GINs produce a factor(s) that promote eosinophil migration in vitro Wildblood et al. (2005). Yacob et al. (2008) reported the presence of an early and high eosinophilia and migration of the same cellular components into the abomasal and intestinal mucosa in the absence of nematodes in the gut. Many studies have been done on this parasite. Ahmedamir et al. (2007), Zacharias et al. (2008), Scott et al. (1999) are reviewed pathophysiology of haemonchosis. Also Ameen et al. (2006), Wilson et al. (1996) reported hematologic changes. Nayebzadeh et al. (2005), Troell et al. (2005) studied at parasite growth stages and its changes. In Anderson (2000) studies, weight loss is reported during the chronic infection with this parasite in sheep. Zacharias

et al. (2008) reported anemia in merino sheep. Also, Courtney et al. (1984), Nayebzadeh et al. (2005) reported it too. Additionally in microscopic examination by Nayebzadeh et al. (2005), proliferation of macrophages and fibroblasts and infiltration of lymphocytes and eosinophils have seen around the adult worms in pyloric region. Moreover, in another study by Scott et al. (1999), fundus region thickening was seen, that caused by mucous cells hyperplasia. Also Mir et al. (2007) reported that lymphocytes from both naive lambs and immune sheep responded to a similar spectrum of molecular and also this report therefore supports the findings of Anthony et al. (2007, 2008) who had suggested that lymphocyte proliferative responses in naive sheep were important in innate resistance to this parasite weight fraction in soluble antigen. These observations suggest that the chronic nature of primary *H. contortus* nematode infections may be due to a change in the type of immune response induced by larval and adult nematodes and that the adult nematodes may actively subvert the initial immune response induced after infection.

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