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# Histopathological Study of *Haemonchus contortus* in Herrik Sheep Abomasum

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## Abstract

Hemonchosis is a very common disease in small ruminants caused by *Haemonchus contortus*, a blood sucking parasite causing anaemia that may be fatal particularly to young animals. The present study was conducted to determine the prevalence of *Haemonchus contortus* in slaughtered sheep's at Urmia abattoir located in the North West of Iran. A total of 2421animals were slaughtered and examined from July 2010 to July 2011 in Urmia abattoir. In case of sheep, 225 out of 2421 were positive and prevalence of *Haemonchus contortus* infestation was 9.3%. Sex wise prevalence of *Haemonchus 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 ($p>0.05$).

**Keywords:** Abomasal; Haemonchus; Sheep; Urmia; Statistical

## Introduction

Abomasum is one of the most important sites for living bursate nematodes belonging to *Trichostrongylidae* family in small ruminants, because it is the site location for 3 pathogen species of GI nematodes e.g., *Haemonchus spp., Teladorsagia spp., Ostertagia spp.*and *Trichostrongylus spp*., meanwhile it was shown that gastrointestinal nematodes could be harmful to the health mortalities, reduce weight gain and other production losses [1,2]. Small ruminants play an important role in maintaining family stability by providing meat, milk, skin and wool, earn cash income and play traditional social and religious roles. Health disorders in all classes of small ruminants represent the major problems and largely on the economics of sheep and goat production [3]. The gastrointestinal nematode, *Haemonchus contortus* (Barber's pole worm), is a major pathogen of ovine throughout the temperate and tropical regions of the world and is a significant cause of production losses. Debilitating infection with this parasite is most commonly seen in young animals while resistance to infection develops, with exposure, in older ovine [4]. Female worms are 18-30 mm long and are easily recognized by the 'barbers pole' appearance of the white ovaries and uteri twisting for the length of the worm around a red blood-filled intestine and males are 10-20 mm long and uniformly reddish-brown [5]. This parasite is a gastrointestinal nematode of special importance in associated with to small ruminant production in warm climates [6] and is a blood feeding nematode which parasitises the abomasum and presents a serious constraint to ovine production in areas with predominantly summer rainfall [7]. The fourth larval (L4) and adult stages of this worm suck blood and in addition, move and leave wounds that hemorrhage from the abomasal wall of the host. A blood sucking *H.contortus* can suck about 0.05 ml blood per day in ovine [8]. *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 [9] and the diagnosis of *Haemonchosis* is usually based upon clinical signs and fecal examinations. Eggs are found in feces when the damage has already been done. So ELISA enables detection of sub clinical infection [10]. Fayza Ahmed Omer et al. [11] showed that histopathological changes of the abomasum were of severe mucosal and submucosal haemorrhages. Epithelial cells indicated degeneration and other revealed hypertrophy. Gastric glands domonstrated some changes and contained, mononuclear cells dominated with eosinophils. Terefe et al. [12] observed that sheep infected with *Oestrus ovis* and *H. contortus* in a natural way eliminated smaller quantities of eggs in feces than those infected with only *H. contortus*. Previous infection with *O. ovis* in the nasal cavity also reduces the development and fertility of *H. contortus* in the abomasum. This reduction is associated with eosinophilia, an increase in abomasal eosinophils and globular leukocytes, as well as an inflammatory response in the abomasal mucosa [12,13]. Among the diseases that impose the survival and productivity of ovine and goats, gastrointestinal nematode infection ranks highest on a global index, with Barber's pole worm being of overwhelming importance [14]. The objective of this study was to histopathological study of *Haemonchus contortus* in Herrik sheep abomasums.

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## 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 150-350mm. 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

During the period from July 2010 to July 2011, a total of 2421Herrik sheep ranging from 2 to 5 years of both sexes were subjected to a careful post-mortem examination during abattoir inspection in Urmia slaughter houses in West Azarbijan province (coordinates: 37°33N, 45°04E) Iran. The Herrik 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 investigation

Abomasa of total 2421 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 3h of slaughter. The abomasum was slit open and their contects 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-HC1solution for 10 h at 37°C. The digest was concentrated over a 400-meshsieve (aperture diameter 51 pm) under a water-jet and the entire sieve contents examined [15,16].

### Histopathological examination

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 hours), after stabilizing embedded in paraffin, sectioned at 5 µm 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 [17].

## Results

### Overall prevalence of *H. contortus* in sheep

In this study, 225abomasa (9.3%) were infected with *Haemonchus 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 2421 sheep revealed that acute and chronic forms of *haemonchosis* are common in the region. And pre acute form of infection has no sign of presence or is utterly rare in above-mentioned region (Table 1, 2 and Figure 1).

### Macroscopic and microscopic findings

Petechial hemorrhage in the abomasal mucosa (Probably due to the attachment of the parasite) (Figure 2), extensive mucosal hemorrhage, inflammation, mucous secretions around lesions and paleness of internal organs were seen (Figure 3). Also abomasal contents were fluidal and partially covered with free blood; the carcasses were paled and have 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

| 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 | 202.2 | 20.8 | 223.0 |
| Monthly Summary (%) | 99.1% | .9% | 100.0% |
| 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 Count | 171 | 11 | 182 |
| Expected Count | 165.0 | 17.0 | 182.0 |
| Monthly Summary (% | 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 Count | 271 | 7 | 278 |
| Expected Count | 252.0 | 26.0 | 378.0 |
| 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 1:** Abomasum monthly contamination with *Haemonchus contort*.

| 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%)** |

**Table 2:** Abomasum seasonal contamination with *Haemonchus contortus*.

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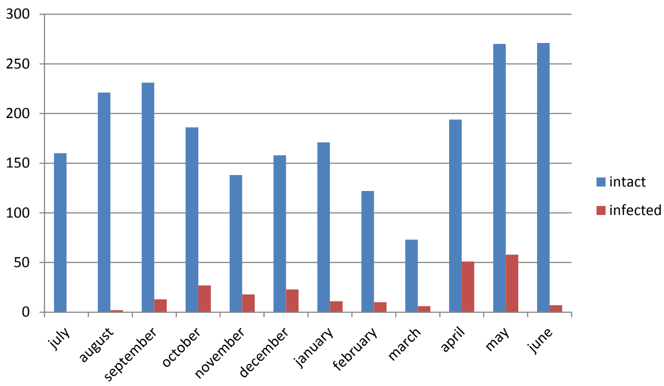


**Figure 1:** Abomasum monthly contamination with *Haemonchus contortus*.

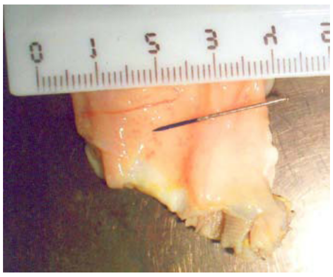

**Figure 2:** Petechial in the mucosa of the abomasum.

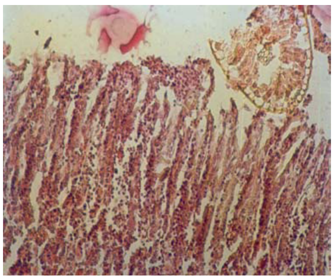

**Figure 3:** hemorrhage in between the glands and inflammatory cell infiltration.

and some even penetrated to the sub mucosa (Figure 4), perivascular hyperemia and lymphocytic infiltration, tissue thickening due to an increase in mucous glands and mucous secretion (Figures 5 and 6), connective tissue proliferation and necrosis (in chronic abomasal inflammation and wound healing) (Figure 7).

## 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 to 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).

## Discussion

*Haemonchosis* is a serious health problem which causes lower production due to high morbidity, mortality, and cost of treatment and control measures [18]. 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 [19]. 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 et al. [19] 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 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

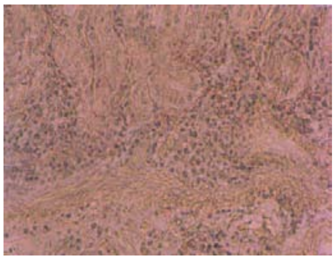

**Figure 4:** Infiltration of inflammatory cells in the mucosa of the abomasum.

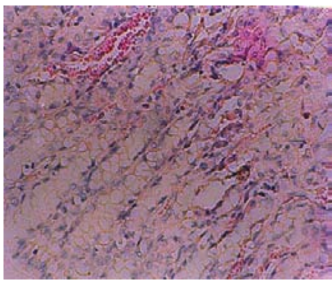

**Figure 5:** Increase in mucous glands and hemorrhage in between the glands.

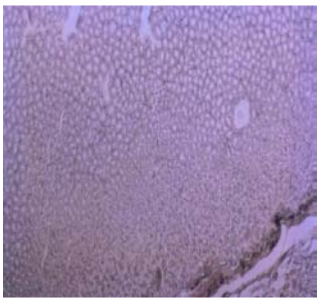

**Figure 6:** Hyperplasia and cystic glands in the abomasum.

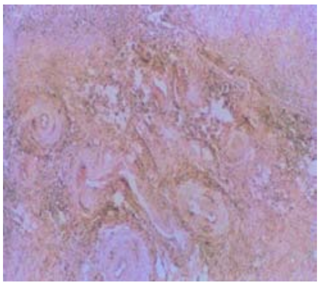

**Figure 7:** Proliferation of Connective tissue.

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 Blitz and Gibbs 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 IO-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 [20-22]. In Macroscopic findings, petechial hemorrhage in the mucosa and severe hyperemia in mucosal folds were seen as described by McKenna PB [21]. Also eosinophilic infiltration has been observed that while corresponding to those of Nayebzadeh et al. [22]. Eosinophils are considered to be important elements in the response against Haemonchus infections Balice et al. [23]. In the study, there was an increased number of circulating blood eosinophils in the sheeps infected with *Haemonchus* larvae. This was in agreement with Terefe et al. [12]. There is also some evidence suggesting that eosinophils may contribute to pathogenesis during parasitic infection [13]. Moreover, it has previously been shown that a number of ovine parasitic gastrointestinal nematodes produce a factor(s) that promote eosinophil migration in vitro [24]. Yacob et al. [25] 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. Meanwhile, sometimes the focal accumulation of lymphocytes and tissue thickening were seen that is similar to that reported by Scott et al. [26]. Many studies have been done on this parasite. Ahmed Mir et al. [27], Zacharias et al. [28] and Scott et al. [26] have reviewed pathophysiology of *Haemonchosis*. Also Ameen et al. [29] and Wilson et al. [30] reported hematologic changes. Nayebzadeh et al. [22] and Troell et al. [31] studied at parasite growth stages and its changes. In Anderson [32] studies, weight loss is reported during the chronic infection with this parasite in sheep. Zacharias et al. [28] reported anemia in merino sheep. Also, Courtney et al. [33] and Nayebzadeh et al. [22] reported it too. Additionally in microscopic examination by Nayebzadeh et al. [22] proliferation of macrophages and fibroblasts and infiltration of lymphocytes and eosinophils have seen around the adult worms in pyloric region [22]. Moreover, in another study by Scott et al. [26], fundus region thickening was seen, that caused by mucous cells hyperplasia. Also Mir et al. [15] 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. [34,35] who had suggested that lymphocyte proliferative responses in naive sheep were important in innate resistance to this parasite weight fraction in soluble antigen. Immunosuppresion is known to take place in some gastrointestinal nematode infection models [36-38]. 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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