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Original Article

Parasitic Burdens, Egg Output, Hemathologic, and Biochemical Changes in Naturally Infected Lambs with *Dicrocoelium dendriticum*

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Abstract

Background: We investigated the variations of hemathologic and biochemical parameters with different parasitic burdens and the correlation between fecal egg counts and fluke number in livers of sheep naturally infected with *Dicrocoelium dendriticum*.

Methods: In order to detect excretion of *D. dendriticum* eggs, fecal samples from 120 sheep of different age groups were collected individually at slaughterhouse in Neyshabur County in Razavi Khorasan Province during December 2013 - October 2014. Hematologic and blood biochemical values in 120 sheep naturally infected with *D. dendriticum* were compared with equivalent values in 120 parasite-free sheep from an organically farmed flock. Investigated animals were kept in outdoor system, on pastures covered with swamps, which remain flooded after rainy season.

Results: Total numbers of flukes in livers obtained from infected sheep at autopsy varied between 10 and 18,500. A positive relationship was noted ($r= 0.94$, $P < 0.001$) between the number of eggs excreted by each sheep and that of total fluke counts in the liver. Significantly, lower red blood cell, packed cell volume, neutrophil and albumin were observed in parasitized sheep, when compared to the healthy animals. White blood cells, lymphocyte and aspartate aminotransferase values were significantly higher in parasitized sheep. No significant correlation was detected between the hematologic and biochemical parameters and the number of *D. dendriticum* in the liver.

Conclusion: Infection with > 400 *D. dendriticum* in the liver could be diagnosed by egg per gram. However, there was no relationship between the parasite burden and hematologic and biochemical parameters.

Introduction

Dicrocoeliosis is a little-known parasitic disease that is not often apparent to the farmer but is of considerable economic and public health importance. This parasite lives in the bile ducts and gall bladder of domestic and wild ruminants throughout the world including Iran (1-3). The life cycle of *D. dendriticum* is complex because it involves two intermediate hosts (land molluscs and ants). Infection of the final hosts occurs by ingesting the ants that harbor infective metacercariae. Dicrocoeliosis produces mild signs in affected animals; however, it causes severe economic losses because of low production of meat and milk due to liver destruction (4).

Dicrocoeliosis often remains undetected or undiagnosed because of its subclinical nature. Its diagnosis is mainly based on recovering adults in the liver on post-mortem examination or detecting eggs at fecal examination. Fecal examination is the simplest and most commonly used technique for the diagnosis of dicrocoeliosis. However, the egg output rate can be influenced by several factors (5,4) and the number of eggs passed into the feces varies from day to day (6,7). Although *Dicrocoelium* egg counts were used to assess the fluke burden in sheep, there are conflicting data on the relationship between *Dicrocoelium* egg output and parasite burden in sheep (8-10). Previous studies explored parasite burden and EPG during single experimental infections, which eliminate all factors except the number of metacercariae. However, natural infections are more complicated, with several dependent factors such as simultaneous mixed infection with gastrointestinal nematodes or feed deficiencies.

Therefore, to evaluate and compare the egg output and parasite burden and hematologic and biochemical findings between *D. dendriticum* naturally infected and non-infected sheep flocks, we conducted this study.

Materials and Methods

This study was conducted in Neyshabur County in Razavi Khorasan Province, which is located in the Northeastern part of Iran. The study area has a generally Mediterranean climate with the rainy seasons mostly in the spring (April-June) and winter (February-March).

During Dec 2013 - Oct 2014, rectal fecal samples from 120 sheep of different age groups were collected individually at a slaughterhouse and transported to the laboratory in polythene bags for examination. Blood samples were taken from the jugular vein into evacuated EDTA tubes and were analyzed for hematologic parameters within 12 h. Blood was also drawn from jugular vein into serum separating tubes, and separated sera were frozen in plastic tubes at -20°C . After slaughter, the liver from each animal was collected and examined in the laboratory for the parasite.

In the laboratory, the livers, gall bladders and feces were subjected to thorough investigation for the collection of parasites and parasitic materials. Animals that had a mixed infection of *D. dendriticum* and *Fasciola spp.* and other parasites in their livers, carcasses and blood were excluded from the study. The animals with liver dicrocoeliosis and no other pathology and parasite in the liver, carcass and blood samples were selected as parasitized group. The negative control animals did not show any pathology and parasite including, *D. dendriticum* in the liver, carcass and blood samples.

The number of *D. dendriticum* eggs was determined by a flotation-centrifugation method according to modified McMaster technique using MgSO_4 solution (1.28 specific gravity). Flukes were recovered from the livers and counted (11).

Total white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were determined using an automated blood cell counter (Nihon kohden, Celltac α , Tokyo, Japan). Serum activities of total protein (TP), albumin, aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) were measured according to standard procedures using an automatic analyzer (Biotechica, Targa 3000, Rome, Italy) with commercial kits (Pars Azmoon, Iran).

Hematologic and biochemical parameters were compared among sheep with different burdens of parasites (0, 10-400, 401-1000 and 1001-18500) by one way ANOVA followed by Games – Howell and Bonferroni post hoc test.

The relationship between fecal egg count, total fluke count and gall bladder fluke counts was analyzed using Spearman's correlation test. All analyses were carried out using SPSS software version 21 and $P < 0.05$ considered as significant.

Results

All of the inspected lambs contained worms in their livers when slaughtered. The Total number of flukes in livers of parasitized sheep varied between 10 and 18,500. Sheep distribution according to parasite burden was as follows: control (no parasite) Group A 10–400; Group B 400–1000; Group C $> 1,000$.

The parasitized lambs, the egg excretion in feces from the parasitized lambs was generally low, and the minimum and maximum eggs per gram (EPG) values were 0 and 50, respectively. Median, first and third quartile of EPG values at different liver intensity group are shown in the Table1. The median EPG at group control, A, B and C was 0, 0, 3.5 and 22, respectively. EPG in group B and C was greater than control and group A ($P < 0.001$). In addition, EPG in group C was more than group B ($P < 0.001$).

Table 1: Liver fluke counts and fecal egg count, gall bladder egg count in naturally infected lambs with *Dicrocoelium dendriticum*

Liver fluke counts	0			1-400			401-1000			>1000		
	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3
Fecal egg count	0	0	0	0	0	0	3.5	2	5	22	10	40
Gall bladder egg count	0	0	0	3100	1600	6000	16000	13040	28400	58000	45000	80000

A positive relationship can be seen via the correlation coefficient ($r = 0.94$, $P < 0.001$) between the number of eggs excreted by each sheep and that of total fluke counts recovered. With the increase in the total fluke counts, an increase was also observed in the number of the gall bladder egg count which this relationship was statistically significant ($r = 0.98$, $P < 0.001$). A statistically significant correlation was also found between fecal egg counts and gall bladder egg counts ($r = 0.85$, $P < 0.001$).

Significantly lower RBC, PCV, neutrophil and albumin and higher WBC, lymphocyte and AST values were recorded in the parasitized sheep, when compared to the healthy

animals ($P < 0.05$) (Table 2). Fibrinogen in group C was significantly lower than control group ($P = 0.023$) (Table2).

Discussion

Few studies have been carried out on dicrocoeliosis under natural conditions in comparison with experimental infection. Experimentally infected sheep are much more sensitive to parasite infections when being kept on pasture without any supplemental feed whereas sheep in conservative systems, fed on diets higher in protein, can improve their immunity to decrease the parasite entrance.

Table 2: Hematologic and biochemical parameter in naturally infected sheep with different doses of *Dicrocoelium dendriticum*

Liver fluke counts								
Variable	0		1-400		401-1000		>1000	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Albumin (g/l)	4.34	0.38	3.77	0.56	3.72	0.57	3.95	0.89
AST (U/l)	43.70	8.56	63.65	13.58	78.33	16.65	86.31	43.65
GGT (U/l)	83.70	13.23	123.24	24.81	134.45	54.71	178.44	44.67
Globulins (g/l)	0.71	0.08	0.59	0.05	0.58	0.045	0.58	0.03
Total protein (g/l)	7.31	0.66	7.23	0.87	7.06	0.85	7.01	0.93
Fibrinogen(g/l)	389.80	179.40	354.55	164.11	345.45	153.46	262.50	114.75
PCV (l/l)	38.35	5.82	22.05	8.64	22.62	7.96	22.45	9.07
Hb (g/l)	10.86	8.35	8.23	3.05	8.55	3.02	8.47	3.14
RBC (×1012/l)	9.93	2.85	7.68	3.03	7.78	3.04	7.77	3.38
MCV (fl)	28.43	3.21	29.05	2.27	29.80	2.64	29.41	2.63
MCH (pg)	10.32	2.34	12.30	6.62	11.20	1.64	11.35	1.45
MCHC (g/l)	34.26	5.46	37.53	6.20	37.86	3.09	38.31	3.99
WBC (×109/l)	865.48	263.16	1292.57	512.64	1195.45	459.17	1185.13	440.89
Lymphocytes (×109/l)	7.60	1.87	4.76	1.37	4.76	1.37	4.76	1.37
Neutrophil (×109/l)	0.53	1.78	0.31	1.45	0.27	1.34	0.19	1.24
Eosinophils (×109/l)	0.01	0.02	0.02	0.03	0.02	0.01	0.03	0.02

Consequently, information obtained from experimental infections in conventionally farmed sheep is may not be applicable in those naturally farmed. In this study, all of sheep in all groups were infected with common gastrointestinal nematodes but fecal tests confirmed that FEC were below the economic threshold (<10).

Different researcher tried to found a relationship between fecal egg and total fluke counts with a linear regression equation (8, 10, 12, 13). Mean number of adult flukes could be deducted from the mean number of eggs for a group of sufficient size (8, 13). The regression equations for estimating the fluke numbers from fecal egg counts have revealed substantial limitation (10). A double logarithmic regression equation fitted best to explain the relationship between fecal egg and total fluke counts based on fecal examinations per sheep before autopsy (12). In our study, although a positive correlation was found between fecal egg counts and total fluke counts, a reliable linear regression equation could not be established for estimating the fluke numbers from fecal egg counts. This could be because the fluke numbers per sheep were low and the egg output was highly variable. Thus, in order to

establish decisively the type of relationship between fecal egg counts and fluke burden, it is recommended to perform further studies using animals infected with a higher infection rate. Therefore, the variations established here should be taken into consideration in future studies when assessing the results of fecal diagnosis in dicrocoeliosis.

We detected significant differences as regards the EPG between the 3 groups of sheep infected with different burdens. This was in difference with Chandra (14) and Tarry (15). In spite of having, collected 1-400 mature worms in-group A, we never observed eggs in their feces. Although egg elimination may have occurred, the number may have been so low that they were not detectable using the normal fecal techniques. The numbers of EPG that we obtained in the sheep infected with 400-1,000 *D. dendriticum* (3.5 EPG) and those infected with more than 1,000 (22 EPG) were much lower than those observed in sheep experimentally infected with 3,000 (347.2 EPG), 1,200 (39.9 EPG) and 1,000 *D. dendriticum* metacercariae (194.8 EPG) (15,9).

Based on our findings, total numbers of flukes in livers obtained in infected sheep var-

ied between 10 and 18,500. The parasitic burden is very variable in naturally infected sheep, with values from 10 worms per animal (12) to 50,000 (16).

In our study, significantly lower RBC, neutrophil, PCV, and albumin, and significantly higher WBC, lymphocyte and AST values were observed in the parasitized sheep, compared to the healthy animals. Hypoalbuminemia is commonly reported in affected sheep (13,17-19); albumin loss may be caused by direct mechanical irritation of bile duct, possibly on account of the suckers of the adult flukes (17). A small increase in albumin and no alteration in the values of total proteins have been reported in lambs experimentally infected with 3,000 *D. dendriticum* metacercariae (20). "Moreover, in naturally infected sheep burdens of up to 4000 *D. dendriticum* worms do not cause significant blood or plasma protein loss" (21). Furthermore, lymphocytosis in the parasitized sheep could be attributed to chronic antigenic stimulation. Neutropenia in parasitized sheep might occur because of enhanced neutrophil consumption in the inflamed tissue and/or diminished granulopoiesis in the bone marrow. Finally, the increase of AST activity in parasitized sheep may be to the result of hepatic parenchymal damage by adult flukes and/or erythrocyte destruction as evidenced by decreased erythrocyte parameter (PCV and RBC).

Estimation of the severity of disease and the prognosis for recovery of affected animals cannot be made on the basis of hematologic and biochemical blood parameters as there was no significant correlation between the number of *D. dendriticum* in the liver of infected sheep, or of hematologic and biochemical parameters in naturally infected sheep.

Infection with more than 400 *D. dendriticum* in the liver could be detected by EPG.

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