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Haematological Parameters and its Relationship with Faecal Egg Count and FAMACHA© Score in *Haemonchus contortus* Naturally Infected Goats

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ABSTRACT

Haemonchus contortus is considered as main gastrointestinal parasite causing anaemia and hypoproteinemia in ruminants. The aim of the present study was to determine the correlation between haematological parameters with duo of faecal egg count and FAMACHA© score in goats predominantly infected with *Haemonchus contortus*. A total number of 100 goats were divided into five groups [Group I (EPG 100-300), II (EPG 400-600), III (EPG 700-1000), IV (EPG 1100-1200) and V (EPG 1300 and above)]. Results revealed a highly significant (P<0.01) negative correlation between EPG and FAMACHA© score with haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC). Mean values of Hb, PCV and TEC were declined in groups with increase in EPG and highly significant (P<0.01) differences were observed between all groups. Highly significant (P<0.01) and negative correlations were observed between TLC, lymphocyte, monocyte with duo of EPG and FAMACHA© scores whereas correlations between EPG, FAMACHA© score with both of neutrophil and eosinophil were highly significant (P<0.01) and positive. However, EPG and FAMACHA© score with basophil were showed significant (P<0.05) and negative correlation. The mean values of TLC were declined in groups having higher worms load. In DLC, the mean values of both neutrophil and eosinophil were elevated but lymphocyte and monocyte values were decreased with the increase in worm burden. Significant (P<0.01) differences were observed in values of TLC and DLC between all groups but, no significant difference was observed for monocyte between group-I and II and for basophil between group-II and IV; and group-III and V.

HIGHLIGHTS

- The paleness of eye conjunctiva showed highly inverse correlation with Hb, PCV and TEC.
- FAMACHA© chart scoring method can be used by farmers to identify anaemia in goats.

Keywords: *Haemonchus contortus,* egg per gram, FAMACHA© score, correlation, haematological parameters

India possesses 135.17 million goats and the total goat contributes around 26.40% of total livestock population (Livestock census, 2012). To boost the economy of developing countries like India goats have enormous potential and can be major source of income especially to marginal farmers and landless laborers. However, goats are very prone to various parasitic diseases that not only undermine their health but also play an important role in lowering the overall production. These parasitic diseases are responsible for reduced production, morbidity and mortality in animals and ultimately causing heavy

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losses especially in developing countries (Singh *et al*., 2015). Commonly occurring gastrointestinal parasitism in goats and sheep are Haemonchosis, Strongyloidosis, Oesophagostomiosis, Bunostomiosis, Trichostrongylosis and Coccidiosis. Among these gastrointestinal parasites, *Haemonchus contortus* is the most prevalent and important parasite (Khalafalla *et al*., 2011). *Haemonchus contortus* is the main culprit causing anaemia and hypoproteinaemia in ruminants. Goats are born without *Haemonchus contortus*, however, the infective (L_3) larvae are eaten by the goats during grazing and they become infected (Bala *et al*., 2015). Depending on level of parasitic load the degree of infection may be sub-clinical or clinical. Sub clinical infections remain dormant and as such are not recognized by the clinicians and owners. These are responsible for a number of economic losses (Fikru *et al*., 2006). For the rational designing of the effective preventive and control measures against these dreadful gastrointestinal parasitic diseases a proper understanding about the epidemiology of gastrointestinal parasitism is mandatory (Rajarajan *et al*., 2017).

In view of the above, the present study was undertaken to determine the correlation between haematological parameters with duo of faecal egg count and FAMACHA© score in goats predominantly infected with *Haemonchus contortus*.

MATERIALS AND METHODS

Selection of animals

Goats of either sex slaughtered at slaughter house located at "Cantonment Board, Mhow" were screened which were positive for more than 80% *Haemonchus contortus*, were taken in our study. A total number of 100 goats predominantly infected with *Haemonchus contortus* were divided into five groups (Group I [EPG 100-300], II [EPG 400-600], III [EPG 700-1000], IV [EPG 1100-1200] and V [EPG 1300 and above]) on the basis of egg per gram (EPG). Total period of study was for 9 months from July 2018 to March 2019.

Collection of blood

Ten ml of blood sample was collected aseptically from goats during slaughter in the blood collecting vial. Then 3-4 ml of blood was transferred to blood collecting vial containing EDTA @ 2mg/ml of blood (anticoagulant) for haematological study and remaining blood sample transferred to sterilize glass test tube without anticoagulant for separation of serum from the blood.

Haematological parameters

The haematological parameters viz. haemoglobin concentration (Hb), packed cell volume (PCV), total Erythrocyte Count (TEC), total Leukocyte Count (TLC) and differential Leukocyte Count (DLC) were carried out as per the standard procedure (Jain, 1986).

Examination of faecal sample

Approximately 5 g of faecal sample was collected directly from rectum of each animal in labeled polythene bags. Qualitative examination of the feacal samples were done by modified Sheather's Sugar floatation technique (Soulsby, 1982) for the detection of nematode eggs. Quantitative examination by Mc-Master's counting technique (Sloss *et al*., 1994) was used for determining egg per gram of faeces. The faecal samples positive for strongyle eggs, were pooled and subjected to copro-culture in separate glass tumblers of 300 ml capacity as per the standard procedure to harvest the infective stage larvae (L_3).

FAMACHA© chart scoring

FAMACHA© eye colour chart clearly depicts various categories from healthy to severely anaemic condition. The ocular mucous membrane of the eye of each goat was examined by comparing them with the laminated color chart bearing the picture of goat conjunctiva (Bala *et al.*, 2015). This chart was calibrated into five categories i.e. 1 = red (non-anaemic); 2 = red-pink (non-anaemic); 3 = pink (mildly-anaemic); 4 = pink-white (anaemic) and 5 = white (severely anaemic). All scorings were done on the same day along with faecal and blood samples.

RESULTS AND DISCUSSION

A total number of 100 goats predominantly infected with *Haemonchus contortus* were divided into five groups (Group I, II, III, IV and V) on the basis of egg per gram (EPG) and subjected to the statistical analysis

employing Pearson correlation analysis between EPG and FAMACHA© eye scores with haematological parameters; and analysis of variance (ANOVA) employing completely randomized design (CRD) (Snedecor and Cochran, 1994). The values were shown at 1%, and 5%, level of significance. Evaluation of analysis was done as: P<0.01, as a highly significant difference; P<0.05, as a significant difference and P>0.05, as a non significant difference.

Grouping of goats

Faecal samples of goats were collected from slaughter house situated at Cantonment Board, Mhow and were checked for the presence of *Haemonchus contortus* infection. A total of 100 goats predominantly infected with *H. contortus* were selected for this study.

Animals were divided into five groups on the basis of egg per gram (EPG) as indicated in Table 1.

Haematological parameters

Blood plays an important role in the general metabolism of the body. Packed cell volume, haemoglobin and mean corpuscular haemoglobin are major indices for the diagnosis of anaemia and are useful to monitor the capacity of bone marrow for erythropoiesis (Chineke *et al.,* 2006).

In the present study, correlation analysis of egg per gram (EPG) with haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and FAMACHA© score were -0.918, -0.851, -0.954 and 0.958, respectively (Table 2). Highly significant (P<0.01) and negative correlation was observed between EPG with Hb, PCV and TEC, whereas highly significant (P<0.01) and positive correlation was observed between EPG and FAMACHA© score. However, correlation of FAMACHA© score with Hb, PCV and TEC was -0.868,-0.807 and -0.917, respectively (Table 2). Highly significant (P<0.01) and negative correlation was observed between FAMACHA© score with Hb, PCV and TEC.

The findings of the present study are in accordance with the findings of Bala *et al.* (2015). They observed highly significant negative correlation between EPG and PCV, EPG and Hb, and positive correlation between FAMACHA© score and EPG.

Mean values of haemoglobin in group-I, group-II, group-III, group-IV and group-V were 11.40±0.18, 10.40±0.09, 9.40±0.13, 7.00±0.28 and 5.40±0.33 g/dl, respectively

Table 1: Grouping of goats on the basis of egg per gram (EPG)

Attributes	Groups				
	I	II	III	IV	V
Egg per gram (EPG)	100-300	400-600	700-1000	1100-1200	1300 and above
Number of Goats	11	20	50	10	9
FAMACHA© chart	Red (non-anaemic)	Red-pink (non-anaemic)	Pink (mildly-anaemic)	Pink-white (anaemic)	White (severely-anaemic)
FAMACHA© scores	1	2	3	4	5

Table 2: Correlation of egg per gram (EPG) and FAMACHA© score with haematological parameters (n=100)

Attributes	Egg per gram (EPG)	FAMACHA© score
	Pearson correlation	
Egg per gram (EPG)	1	0.958**
Haemoglobin (Hb) (g/dl)	-0.918**	-0.868**
Packed cell volume (PCV) (%)	-0.851**	-0.807**
Total erythrocyte count (TEC) (million/cu.mm)	-0.954**	-0.917**
FAMACHA© scores	0.958**	1

**Correlation is significant at the 0.01 level (P<0.01).

(Table 3). Highly significant (P<0.01) difference in mean values were reported between all the groups and these values were gradually decreases with the increasing worms load in different groups. Similar finding was also reported by Bordoloi *et al.* (2012) whereas, Bala *et al.* (2015) observed relatively non-significant lower values of Hb in infected goats.

Mean values of PCV in group-I, group-II, group-III, group-IV and group-V were 35.30±0.66, 31.60±0.69, 30.00±0.44, 22.90±1.14 and 15.00±0.90 %, respectively (Table 3). A highly significant (P<0.01) difference was observed in mean values of PCV between different groups.

The mean values of PCV were gradually decreased with the increasing numbers of EPG in different groups. Similar observation was also reported by Bala *et al.* (2015). FAMACHA© and PCV demonstrated to be good indicators of Haemonchosis, having moderate to high correlations with EPG (Rodríguez *et al*., 2015).

Mean values of TEC in group-I, group-II, group-III, group-IV and group-V were 16.00±0.27, 13.90±0.16, 11.80±017, 8.40±0.35 and 6.40±0.37 million/cu.mm, respectively (Table 3). Highly significant (P<0.01) difference in mean values of TEC was observed between all the groups. The values of TEC were gradually decreased with higher worms load in different groups. Similar finding was also reported by Bordoloi *et al.* (2012); and Qamar and Maqbool (2012).

Hematological studies revealed decreased values of Hb, PCV, and TEC counts in goats in relation to *Haemonchus contortus* parasitic infection. The reduction in the values of these components in infected animals may be due to loss of blood during piercing of mucosa by the parasite and also by feeding of the worms, as both L_4 and adults are haematophagous. Average blood loss due to *H. contortus* infection has been reported to be 0.05 ml per day per worm (Urquhart *et al*., 2000). Abdel (1992) described that the reduced values Hb, PCV, and TEC count in infected groups may be attributed to the bleeding of abomasums due to the injuries caused by the *Haemonchus* parasites.

The correlation between EPG and TLC was -0.986 and the

Table 3: Mean values of haemoglobin, packed cell volume and total erythrocyte count in different groups

Attributes	Groups				
	I	II	III	IV	V
Haemoglobin (Hb) (g/dl)**	11.40±0.18^a	10.40±0.09^b	9.40±0.13^c	7.00±0.28^d	5.40±0.33^e
Packed cell volume (PCV) (%)**	35.30±0.66^a	31.60±0.69^b	30.00±0.44^c	22.90±1.14^d	15.00±0.90^e
Total erythrocyte count (TEC) (million/cu.mm)**	16.00±0.27^a	13.90±0.16^b	11.80±0.17^c	8.40±0.35^d	6.40±0.37^e

**Highly significant (P<0.01); Mean bearing different superscript within a row differ significantly.

Table 4: Correlation of egg per gram (EPG) and FAMACHA© score with leukocyte count (n=100)

Attributes	Egg per gram (EPG)	FAMACHA© score
	Pearson correlation	
Total leukocyte count (TLC) (thousand/cu.mm)	-0.986**	-0.934**
Percent Differential leukocyte count (DLC)		
Neutrophil	0.981**	0.930**
Lymphocyte	-0.742**	-0.668**
Monocyte	-0.746**	-0.799**
Eosinophil	0.873**	0.842**
Basophil	-0.203*	-0.210*

**Correlation highly significant at the 0.01 level (P<0.01); *Correlation significant at the 0.05 level (P<0.05).

correlation between FAMACHA© and TLC was -0.934 (Table 4). Correlations between TLC and duo of EPG and FAMACHA© scores were highly significant (P<0.01) and negative. The correlation study of EPG with neutrophil, lymphocyte, monocyte, eosinophil and basophil were 0.981, -0.742, -0.746, 0.873 and -0.203, respectively (Table 4). Highly significant (P<0.01) and negative correlations were observed between EPG and duo of lymphocyte and monocyte, whereas correlations between EPG and duo of neutrophil and eosinophil were highly significant (P<0.01) and positive. However, EPG and basophil were significant (P<0.05) and negatively correlated.

The correlation study of FAMACHA© with neutrophil, lymphocyte, monocyte, eosinophil and basophil were 0.930, -0.668, -0.799, 0.842 and -0.210, respectively (Table 4). Highly significant (P<0.01) and negative correlations were observed between FAMACHA© score with lymphocyte and monocyte, whereas highly significant (P<0.01) and positive correlations were reported between FAMACHA© score with neutrophil and eosinophil. However, FAMACHA© score and basophil were significant (P<0.05) and negatively correlated.

The mean values of TLC in group-I, group-II, group-III, group-IV and group-V were 13.20±0.24, 11.00±0.22, 8.90±0.11, 7.00±0.22 and 5.10±0.26 thousand/cu.mm, respectively (Table 5). The mean values of TLC decreases in groups having higher worms load and statistically highly significant (P<0.01) differences were reported among all the groups.

In DLC, the mean values of lymphocyte in group-I, group-II, group-III, group-IV and group-V were 61.40±0.66, 58.30±0.31, 52.80±0.36, 48.60±0.66 and 42.10±0.97%, respectively and the mean values of monocyte in group-I, group-II, group-III, group-IV and group-V were 3.00±0.15, 2.80±0.04, 2.40±0.04, 1.80±0.09 and 1.50±0.10%, respectively (Table 5).

These values were decreased in groups having high worms burden and the differences were statistically highly significant (P<0.01) between all the groups except in monocyte in which, no significant difference was observed between group-I and II. The mean values of neutrophil in group-I, group-II, group-III, group-IV and group-V were 30.60±0.54, 33.60±0.24, 38.00±0.26, 42.40±0.51 and 47.20±0.70 %, respectively and the mean values of eosinophil in group-I, group-II, group-III, group-IV and group-V were 3.50±0.15, 4.60±0.13, 5.60±0.07, 6.70±0.16 and 8.10±0.23 %, respectively (Table 5). These values were increased in groups having high worms load and statistically highly significant (P<0.01) differences were found between all groups.

Eosinophilia was observed in goats heavily infected with *H. contortus* and this finding is in agreement with previous reports (Leal *et al.,* 2011; Ortolani *et al.,* 2013). The present study revealed significant (P<0.01) increase of eosinophil and neutrophil by parasite infection. The results are in agreement with the findings of Bhat and Sharma (1990) who concluded that eosinophilia is associated with antigenic stimulation or parasite burden. Similar finding of eosinophilia was also reported by Terefe *et al*. (2005), and Qamar and Maqbool (2012) in *Haemonchus* parasite infections. But Hosseini *et al.* (2012) did not find any significant changes in the eosinophil count in case of *H.*

Table 5: Mean values of leukocytes in different groups

Attributes	Groups				
	I	II	III	IV	V
Total leukocyte count (TLC) (thousand/cu.mm)**	13.20±0.24^{a}	11.00±0.22^{b}	8.90±0.11^{c}	7.00±0.22^{d}	5.10±0.26^{e}
Differential leukocyte count (DLC)**					
Neutrophil (%)**	30.60±0.54^{e}	33.60±0.24^{d}	38.00±0.26^{c}	42.40±0.51^{b}	47.20±0.70^{a}
Lymphocyte (%)**	61.40±0.66^{a}	58.30±0.31^{b}	52.80±0.36^{c}	48.60±0.66^{d}	42.10±0.97^{e}
Monocyte (%)**	3.00±0.15^{a}	2.80±0.04^{a}	2.40±0.04^{b}	1.80±0.09^{c}	1.50±0.10^{d}
Eosinophil (%)**	3.50±0.15^{e}	4.60±0.13^{d}	5.60±0.07^{c}	6.70±0.16^{b}	8.10±0.23^{a}
Basophil (%)**	1.60±0.12^{a}	0.70±0.04^{c}	1.20±0.04^{b}	0.50±0.06^{c}	1.10±0.13^{b}

**Highly significant (P<0.01); Mean bearing different superscript within a row differ significantly.

contortus infection. Increase in the eosinophil count is an indicator of infection by tissue invading helminthes (Soulsby, 1982).

The mean values basophil in group-I, group-II, group-III, group-IV and group-V were 1.60±0.12, 0.70±0.04, 1.20 ± 0.04, 0.50±0.06 and 1.10±0.13%, respectively (Table 5) and found highly significant (P<0.01) difference between all groups except between group-II and IV; and group-III and V.

A significant reduction in the WBC counts was observed in this study and this finding is in agreement with the observation of Leal *et al.* (2011), Qamar and Maqbool (2012) and Ortolani *et al.* (2013) in *H. contortus* infected goats. The decrease is related to the destruction of lymphocytes in lymphoid organs and infiltration of these cells into various organs (Sandhu *et al.,* 1998).

CONCLUSION

The study demonstrates the negative correlation between pallor scoring of ocular mucous membrane with haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC). Treatment should be recommended to goats which come under group 2 and above. FAMACHA© chart score method can be used by farmers to identify anaemic goats particularly in conditions of haemonchosis which is one of the main causes of anaemia in goats and the most predominant gastrointestinal nematode in small ruminants in the study area.

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