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Haematological variations in visually anaemic sheep naturally infected with *Haemonchus contortus* in farm conditions at arid Rajasthan

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ABSTRACT

With an objective to reduce use of anthelmintic frequency, targeted selective treatment was implemented for farm flocks in arid Rajasthan. An eye colour chart developed by CSWRI, Avikanagar was used for screening the flocks at monthly interval from July to March each year (from 2008 to 2016). All the scorings were done on the same day along with collection of faecal and blood samples from visually anaemic sheep and estimated faecal egg counts (FECs) and erythron parameters, respectively. Data generated were used to establish relationship among haematological estimates and intensity of strongyle infection in visually anaemic sheep. Out of 687 visually anaemic sheep, maximum proportion (54.1%) was recorded in monsoon (Jul-Sep). The frequency distribution exhibited a maximum of 36.3% of visually anaemic sheep with high level (>2001 epg) of strongyle infection. The mean intensity of strongyle infection in visually anaemic sheep varied significantly from nil (nil epg group) to 8631.6±491.3 epg (>2001 epg group). A significant influence of strongyle infection level was observed on Hb, PCV and mean corpuscular haemoglobin concentration (MCHC). The overall Hb concentration varied from 5.7 (>2001 epg) to 7.2g% (nil epg). The overall magnitude of PCV exhibited a linear decline with an increased level of infection and varied from 15.7 (>2001 epg) to 20.5% (nil epg). MCHC showed a marginal but significant increase in sheep with >2000 epg compared to other groups. The Pearson correlation coefficient exhibited negative correlation between intensity of strongyle infection and haematological parameters like Hb, PCV and TEC in all FEC levels, but it was significant only in sheep with FEC >2001 epg. Periodic monitoring of intensity of worm infection and status of anaemia in host animals are an important part of parasite management programmes which aim to avoid both serious parasitism and excessive chemical treatments.

Key words: Anaemia, Haematology, *Haemonchus contortus*, Rajasthan, Sheep, Targeted selective treatment

Infection with gastrointestinal nematode parasites is a major constraint to the sheep productivity and cause production losses, increased costs of management and treatment and even mortality in severe cases. Based on sheep population of Rajasthan, Singh *et al*. (2011) estimated an annual loss of ₹ 1191.708 million due to gastrointestinal nematodes (predominated by *Haemonchus contortus*) in sheep flocks. *H. contortus* penetrates the surface of the abomasal mucosa to feed on the blood of the host (Jasmer *et al*. 2007) and each worm sucks about 0.05 ml blood/day by ingestion or seepage from lesions (Urquhart *et al*. 2000). Thus, *H. contortus* causes severe blood loss from the gut with a resultant decline in erythron parameters and sudden death due to haemorrhagic anaemia (Vatta *et al*. 2001). The understanding of the effect of this parasite on haematological parameters is essential to reduce the losses caused by this infection in sheep. Investigations of the pathogenesis of haemonchosis were generally made only over a relatively short period of infection (during acute phase) under experimental challenge. As a result there is little information regarding the haematological variations during different level of infection (sub-clinical to chronic) under natural challenge. Present study was conducted with an objective to reduce use of anthelmintic frequency by identifying visually anaemic sheep in flocks managed under natural challenge of infection in farm condition in arid Rajasthan. The emphasis was on determining the conjunctiva colour scoring by eye color chart and establishing a relationship between the intensity of strongyle infection (predominated by *H. contortus*) and haematological values in visually anaemic sheep.

MATERIALS AND METHODS

The study was conducted in sheep flocks (5 flocks covering an average of 500 sheep each year) maintained under semi intensive system at Sheep Breeding Farm, Fatehpur (Sikar), Rajasthan. The eye colour chart (Singh and Swarnkar 2012) depicting five categories from normal (1, red; 2, red-pink; 3, pink) to anaemic (4, pink-white; 5, white) condition of sheep was used for screening the flocks

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at monthly interval from July to March each year (from 2008 to 2016). All scorings were done on the same day along with collection of faecal and blood samples from visually anaemic sheep (categories 4 and 5). The faecal samples were processed for strongyle faecal egg counts (FECs) by modified McMaster method (MAFF 1986). Predominance of *H. contortus* was established by estimating the generic composition of infective larvae on pooled faecal culture. Blood samples were analysed to estimate haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) by standard procedure (Schalm *et al.* 1975). The weather of study area was divided into three seasons; winter (October to December); spring (January to March) and monsoon (July to September). No observations were taken during summer (April to June). The haematological data from visually anaemic sheep were grouped as per intensity of infection as nil, 100–500, 501–2000 and >2001 epg. The data were analyzed by analysis of variance and means were compared by Duncun's multiple range test. Pearson correlation coefficient was calculated to determine the extent and direction of correlation between FEC and haematological parameters using SPSS ver 15.

RESULTS AND DISCUSSION

Out of 687 visually anaemic sheep, maximum proportion (54.1%) were recorded in monsoon (July – September) followed by 34.2% in winter (October – December) and minimum of 11.6% in spring season (January – March). Like-wise, relatively higher numbers of anaemic sheep were identified from July to October (Swarnkar and Singh 2014). The overall frequency distribution of visually anaemic sheep exhibited 14.1% with absence of strongyle infection, 24.6% with low level (100–500 epg), 23.0% with moderate level (501–2000 epg) and 38.3% with high level (>2001 epg) of strongyle infection (Table 1). Seasonal analysis revealed predominance of sheep with high level of strongyle infection in monsoon and winter. However, during spring, majority of visually anaemic sheep possess low to moderate level of infection. The bioclimatographs for *H. contortus* showed that suitable climatic conditions for translation of exogenous stages of parasites were from July to August in arid Rajasthan (Swarnkar and Singh 2011). Similarly, in arid Rajasthan, Singh and Swarnkar (2013) observed that incidence of strongyle infection started rising with the occurrence of pre-monsoon rain in June (resumption of development in hypobiotic larvae) and remained higher during monsoon and declined thereafter (availability of climatic conditions suitable for translation of exogenous stages of nematodes). Over-dispersed profile of intensity of infection was a common feature in sheep flocks (Hoste *et al*. 2001) and found to vary as per season. In sheep flocks of Rajasthan, it was observed that even during favourable monsoon season (July-September) around 20–30% and 50% of animals in field and farm conditions, respectively had FECs to the tune of >1000 epg. Otherwise, in rest of seasons, majority of animals had either <200 epg or were free of infection (Swarnkar *et al*. 2008). The requirement for treatment is a reflection of the genetic basis of host variation in either innate or acquired resistance to parasites or resilience (Bisset *et al*. 2001).

Table 1. Frequency distribution (%) of visually anaemic sheep as per level of strongyle infection (Predominantly *Haemonchus contortus*)

Factor (n)	Intensity of strongyle infection (epg)			
	Nil	100-500	501-2000	>2001
Overall (687)	14.1	24.6	23.0	38.3
Season				
Jul-Sep (372)	9.4	23.1	21.8	45.7
Oct-Dec (235)	19.6	24.3	21.3	34.9
Jan-Mar (80)	20.0	32.5	33.8	13.7

Based on level of strongyle infection, the overall mean intensity in visually anaemic sheep ranged significantly (P<0.001) from nil (nil epg group) to 8631.6±491.3 epg (>2001 epg group). Within the group, intensity of strongyle infection was significantly (P<0.05) affected by season and that too in only those visually anaemic sheep which possessed high level of infection (>2001 epg) and exhibited around 2.5 to 3 times lower magnitude of infection during January to March as compared to period during July to December (Table 2). The seasonal patterns in parasitism further indicate that the long dry season may limit development and survival of parasite stages in the environment and, as a result, host contact and parasite transmission. Further, host immunity may also vary seasonally in relation to changes in reproduction (Altizer *et al*. 2006), stress, nutrition, and photoperiod (Martin *et al*. 2008) and regulate intensity of infection and its pathogenic effects.

The haematological analysis revealed significant (P<0.001) influence of level of strongyle infection on haemoglobin, PCV and mean corpuscular haemoglobin concentration. The overall haemoglobin concentration and PCV varied (P<0.001) from 5.7 (>2000 epg) to 7.2g% (nil epg) and 15.7 (>2000 epg) to 20.5% (nil epg), respectively with significant influence of season in anaemic sheep possessing low to moderate level of strongyle infection. The effect of infection remained non-significant on total erythrocyte count, mean corpuscular haemoglobin and mean corpuscular volume. However, mean corpuscular haemoglobin concentration showed a marginal but significant (P<0.001) increase in sheep with >2000 epg compared to other groups.

The Pearson correlation coefficient exhibited negative correlation between intensity of strongyle infection and haematological parameters like haemoglobin, PCV and TEC in all FEC levels; however, it was significant (P<0.001/ 0.05) only in sheep with FEC >2001 epg. The Pearson coefficients between FEC and erythrocytic indices were non-significant but an inverse pattern was observed with rise in magnitude of infection (Table 3).

The escalation of anthelmintic resistance in nematode parasites of small ruminants calls for new methods for

Table 2. Mean (±SE) intensity of infection and haematological observations in visually anaemic sheep

Parameter		Groups as per intensity of strongyle infection (epg)			
		Nil	100-500	501-2000	>2001
FEC (epg)					
Overall**		0.0±0.0^{a} NS	261.5±10.3^{b} NS	1113.3±33.4^{b} NS	8631.6±491.3^{c*}
Season	Jul-Sep	0.0±0.0	264.0±13.9	1122.2±49.4	9091.2±639.4^{b}
	Oct-Dec	0.0±0.0	250.1±17.4	1154.0±54.2	8342.7±825.7^{b}
	Jan-Mar	0.0±0.0	276.9±30.0	1011.1±78.1	3681.8±663.6^{a}
Hb (g%)					
Overall**		7.2±0.2^{c} NS	6.8±0.1^{b*}	6.6±0.1^{b**}	5.7±0.1^{a} NS
Season	Jul-Sep	7.7±0.3	7.1±0.1^{b}	6.9±0.1^{b}	5.6±0.1
	Oct-Dec	6.9±0.2	6.5±0.2^{a}	6.2±0.2^{a}	6.0±0.2
	Jan-Mar	6.9±0.4	6.5±0.2^{a}	6.0±0.2^{a}	6.1±0.4
PCV (%)					
Overall**		20.5±0.5^{c} NS	19.7±0.4^{c*}	18.3±0.4^{b*}	15.7±0.3^{a} NS
Season	Jul-Sep	20.6±0.9	20.8±0.5^{b}	19.3±0.5^{b}	15.6±0.4
	Oct-Dec	20.7±0.7	18.5±0.7^{a}	17.7±0.6^{ab}	15.9±0.5
	Jan-Mar	19.9±1.3	19.2±0.7^{ab}	16.7±0.8^{a}	16.3±1.4
TEC ($\times 10^6$/mm^3)					
Overall ^{NS}		3.1±0.2 NS	3.5±0.2 NS	3.2±0.2 NS	3.0±0.1 NS
Season	Jul-Sep	3.6±0.4	3.7±0.3	3.2±0.3	2.9±0.2
	Oct-Dec	3.1±0.3	3.1±0.2	3.4±0.3	3.2±0.2
	Jan-Mar	2.1±0.3	3.8±0.5	3.0±0.5	2.6±0.3
MCH (μ μg)					
Overall ^{NS}		28.9±2.3 NS	25.6±1.6 NS	28.5±1.8 NS	26.1±0.9 NS
Season	Jul-Sep	26.6±3.5	24.6±2.3	30.6±2.9	27.5±1.3
	Oct-Dec	29.7±3.6	28.9±3.3	25.8±2.7	23.7±1.2
	Jan-Mar	31.8±3.9	21.3±1.8	28.5±3.4	26.0±2.7
MCHC (%)					
Overall**		35.8±0.7^{a} NS	35.5±0.5^{a} NS	36.9±0.6^{ab} NS	38.2±0.6^{b} NS
Season	Jul-Sep	38.0±1.4	35.3±0.8	37.3±0.9	37.6±0.7
	Oct-Dec	34.3±1.0	36.4±0.9	36.3±0.9	39.3±1.0
	Jan-Mar	35.7±1.4	34.6±1.4	36.7±1.5	38.6±2.8
MCV (μ^3)					
Overall ^{NS}		80.2±5.4 NS	70.8±4.0 NS	75.2±4.2 NS	68.8±2.4 NS
Season	Jul-Sep	69.2±7.7	68.9±5.3	79.4±7.0	73.2±3.5
	Oct-Dec	86.0±8.6	77.8±8.5	68.0±5.9	60.7±2.8
	Jan-Mar	85.2±8.8	61.7±5.0	78.7±9.1	69.7±6.9

Table 3. Correlation coefficient between intensity of infection and haematological parameters in visually anaemic sheep

FEC level	Hb	PCV	TEC	MCH	MCHC	MCV
100-500 epg	- 0.017	- 0.039	- 0.022	- 0.078	0.012	- 0.093
501-2000 epg	- 0.053	- 0.099	- 0.005	- 0.001	0.090	- 0.039
>2001 epg	- 0.436**	- 0.294**	- 0.213*	0.124	- 0.082	0.164

sustainable management of gastrointestinal nematodes. Among them, targeted selective treatment (TST) using the FAMACHA system has been proposed as an important alternation (van Wyk *et al*. 2006). Periodic monitoring of intensity of worm infection and signs of anaemia in host animals are an important part of integrated parasite management programmes which aim to avoid both serious parasitism and excessive chemical treatments. Although anaemia is the key pathogenic process in haemonchosis and blood loss is closely linked to the *H. contortus* burden (Le Jembre 1995), it is rarely practical or efficient to utilize haematology to confirm a diagnosis or to indicate impending disease (Besier *et al*. 2016). Critical values associated with terminal haemonchosis are evident from field and pen observations - a fall in PCV to <15% for an individual animal is generally fatal, unless immediate treatment is given (Albers *et al*. 1989). Using the perhaps more consistent index of haemoglobin concentration, Roberts and Swan (1982) considered values of <8.5 g% to be indicative of heavy *H. contortus* burdens. Scheuerle *et al*. (2010) reported positive correlations between FEC and eye scores in all study months but significant only in June.

In acute form, there is an anaemia and progressive dramatic fall in PCV, which causes increase in-appetence, exhaustion of bone marrow due to continuous loss of iron and proteins in the gastrointestinal tract and thus resulting into death. In the case of chronic infection with

Haemonchus, the signs are not very obvious although there is a mild drop in the packed cell volumes compared to uninfected sheep (Barger and Cox 1984). PCV as a measure of anaemia due to haemonchosis has been found to be significantly negatively correlated with FEC (Scheuerle *et al*. 2010). Costa *et al*. (2000) and Ejlertsen *et al*. (2006) observed moderate level of correlation (–0.45) between log-transformed FECs and PCVs before any treatment in various goat breeds in Brazil and Kenya. Diaz-Anaya *et al*. (2014) observed no correlation between the presence of the parasite and the haematological parameters. Faecal egg count was negatively correlated with Hb concentration and PCV (Kasali *et al*. 1988, Kelkele *et al*. 2012). Abay *et al*. (2015) reported a highly significant negative correlation to the tune of –0.78 and –0.34 observed between *Haemonchus* larvae and PCV, and PCV and eye color, respectively. The PCV of the *Haemonchus* positive sheep had a strong negative correlation (–0.023) with the FEC. When PCV fell below 19%, FEC were higher than 1050 epg with >2000 epg in the majority (Kaplan *et al*. 2004). Bordoloi *et al*. (2012) reported hypochromic normocytic type of anaemia developed due to experimental haemonchosis in Sahabadi sheep. Yadav *et al*. (1993) observed significantly lower Hb and PCV level in sheep with higher faecal egg count and worm burden of *H. contortus*. Significant decrease in the Hb, TEC and PCV was observed in small ruminants during natural infection of *H. contortus* (Kumari *et al*. 2013). It is well recognized that *H. contortus* worm burdens are highly correlated with levels of anaemia (Le Jambre 1995).

It is concluded that periodic monitoring of intensity of infection and status of anaemia in host animals are an important part of parasite management programmes which aim to avoid both serious parasitism and excessive chemical treatments. By only treating those animals with high infection levels, indicated by the level of anaemia, it would be possible to reduce the number of anthelmintic treatments substantially, leaving refugia of untreated parasites with acceptable level of control.

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