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Comparative Resistance of Sheep Breeds to *Haemonchus contortus* in a Natural Pasture Infection

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ABSTRACT

The objective of the present study was to evaluate the resistance status of three indigenous sheep breeds (Thalli, Lohi & Kachhi) against *Haemonchus contortus* by natural pasture challenge infection. Following deworming, 15 male lambs of each breed were subjected to selected contaminated pastures in a vicinity of the livestock farms. Between breed variation was assessed on the basis of parasitological, hematological, biochemical and cellular parameters. After nine weeks, 10 lambs of each breed were randomly selected and slaughtered to evaluate the differences in adult worm burden among the sheep breeds. Overall, Lohi sheep performed relatively better in the trial; however difference between breeds remained low. Both significant (P<0.05) and non-significant differences with respect to resistance against haemonchosis were noted at different intervals in all the practiced parameters except fecal egg counts, which showed non-significant difference throughout the trial. This report did not provide the major difference among breeds. This type of study on a large scale could be a model in identification of comparatively resistant sires and incorporation in breeding programs for the improvement of flocks concerning resilience and resistance against nematodes that could lessen dependence upon chemical anthelmintics. © 2010 Friends Science Publishers

Key Words: Variability; Natural resistance; *H. contortus*; Sheep breeds; Pasture

INTRODUCTION

Small ruminants play a crucial role in the economy of Pakistan by providing extra income and support survival to poor-resource farmers. Production in small ruminant industry can be affected significantly due to high prevalence of parasitic diseases such as haemonchosis caused by an abomasal nematode of small ruminants - *Haemonchus contortus*. Prevalence of this parasite is very high in Pakistan (Iqbal *et al.,* 1993; Lateef *et al*., 2005) as other tropical and sub-tropical areas globally (Aumont *et al.,* 1997; Notter *et al.,* 2003).

Chemical control of haemonchsosis is becoming less effective due to the development of anthelmintic resistance against most commonly used anthelmintics (Saddiqi *et al.,* 2006; Jabbar *et al.,* 2006; Saeed *et al.,* 2007; 2010). Moreover the demand for organic meat is also increasing worldwide (Thamsborg *et al.,* 1999). In these circumstances, alternative worm control strategies are needed, which may include: grazing management, use of botanical anthelmintics, vaccination and protein supplementation. However there are some limitations in the application of these alternate worm control strategies. For example, communal grazing does not allow proper pasture management; variation in the doses/efficacy of botanical anthelmintics; non-availability of commercial anti-parasitic vaccines and cost factor in nutritional supplementation. In this situation, evaluation/breeding of sheep for resistance against gastrointestinal nematodes (GINs) could be a useful strategy (Mugambi *et al.,* 2005). Relatively resistant animals are known to have better growth, less contamination of the pasture and could be helpful to minimize the development of anthelmintic resistance (Miller *et al.*, 1998; Matika *et al*., 2003).

Pakistan is endowed with 29 purebred and 2 crossbred sheep (Khan *et al*., 2005) with an estimated population of 27.4 million (Livestock Census, 2009). Prevalence of gastrointestinal parasites in sheep ranges from 25.1 to 92% in different parts of Pakistan (Durrani *et al*., 1981; Iqbal *et al*., 1993; Qayyum, 1996). Field observations indicate differences in prevalence rates of parasites among different breeds of sheep in the same environment and under similar management conditions. There is, however no scientific evidence for natural resistance of sheep against parasites. The present study was, therefore undertaken to evaluate three commonly reared breeds of sheep in Pakistan for natural resistance against *H. contortus*.

MATERIALS AND METHODS

Animal characteristics and management: Three indigenous sheep breeds Thalli (TH), Lohi (LH) and Kachh

(KCH) were used in the study. All these animals are thin-tailed mutton breeds ranging from medium to large size. The locale of TH is Thall desert (Punjab), while LH exists in the central Punjab. The natural habitat of KCH is Ran of Kachh (Sindh). Fifteen pure bred 6-months old lambs of each sheep breed, having same lambing week, were selected for the study. Lambs were vaccinated for enterotoxaemia and pleuropneumonia. The experiment was carried out at the livestock farms, University of Agriculture, Faisalabad (UAF), Punjab, Pakistan.

Experimental flock history and design: Animals used in the current study were previously infected with artificial infection of *H. contortus* larvae at 3-months of age. At the start of the trial, all the animals were treated with Avermectin (Inj. Actimec; Sellmore Pharmaceuticals Pakistan) at the dose rate of 1 mL/50 kg live weight (LW) to make them nematode free. Animals were also kept under better feeding conditions to permit recovery from previous infections. Contaminated fecal pallets from the artificially infected lambs were thrown evenly on a fresh pasture (not grazed by the animals, previously) for experiments on natural infection. Fifteen lambs of each breed were used for the studies on natural pasture infection with *H. contortus*. Animals were subjected to contaminated pastures for grazing during the day time for 9 weeks and following grazing animals were kept in a shed on seasonally available green fodder (s) supplemented with concentrate mixtures. The water was available *ad libitum* in shed.

Experimental Parameters

Fecal egg count (FEC), worm count (WC) and coproculture: Fresh fecal samples were taken weekly, directly from the rectum of all experimental animals and analyzed for FEC by modified McMaster technique (Gordon & Whitlock, 1939) in which each nematode egg counted represented 50 eggs per gram of feces. At 9th week post infection (PI), 10 animals of each breed were randomly selected and slaughtered for adult WC. After opening the abdominal cavity, abomasa were removed and opened along their greater curvature. The contents of abomasa were collected in a graduated bucket and an aliquot corresponding to 10% of the content was fixed in 5% buffered neutral formalin for WC. Coproculture was carried out in mid and end of the trial to determine the status of *H. contortus*.

Packed cell volume (PCV) and live weight: PCV was determined at 2, 4, 6, 8 and 9 weeks PI within 2-4 h of blood collection using microhaematocrit centrifugation technique (Preston & Allonby, 1978). Animals were also weighed on an electric balance separately, at 2, 4, 6, 8 and 9 weeks PI in the morning to estimate any change in their live weight.

Total serum protein and albumin: Total serum protein and albumin concentrations (g/dL) were measured on the same days of PCV by colorimetric method using clinical chemistry kits (Wiesbaden, Germany).

Statistical analyses: Differences between breeds in terms of EPG, PCV, total serum protein (TSP), albumin (SA), LW and WC were assessed by analysis of variance using repeated measure design general linear model Statistica version 6 (SAS institute, Cary, NC). The data were analyzed considering the effects of breed and total period (week 1-9). The values of EPG and WC were $\log_{10}(x + 25)$ transformed to stabilize variance. Transformed values of FEC and WC were back transformed and results were presented as arithmetic means (± S.D). Inferences were drawn on the basis of transformed FEC and nematode burden and untransformed haematological and biochemical parameters. The level of significance was set as 0.05.

RESULTS

Fecal egg counts: Response of different breeds to natural *H. contortus* infection was similar. Though FEC values differed numerically, no statistical difference was recorded among different breeds and weeks post-exposure to contaminated pasture. All the sheep breeds started passing eggs in 3rd week PI and FEC increased gradually with ultimate egg counts in 9th week (Table I). Therefore there was an early establishment of *H. contortus* infection in TH followed by KCH and LH sheep. LH was superior in delaying the establishment of *H. contortus* infection. This may be substantiated by the fact that in 7th week post-exposure, FEC declined in LH and KCH sheep compared with 6th week counts; whereas this decline was not observed in TH sheep (Table I). Coprocultures of pooled samples from all the naturally infected breeds indicated 99% contribution of *H. contortus* to the total nematode larvae, with the rest being *Trichostrongylus* species.

Worm count at necropsy: Kacchi and LH sheep showed better resistance status against *H. contortus* as compared to TH as the lowest number of adult *H. contortus* was recovered from KCH sheep followed by LH and TH (Fig. 1). The pattern of WC was different from that of FEC. Numerically higher FEC was recorded in LH; whereas higher number of worms was recovered from KCH sheep.

Packed cell volume: During 4th, 6th and 8th week, haematocrit values differed ($P < 0.05$) among three breeds (Table II). Compared with initial values (2nd week) of heamtocrit, maximum reduction in haematocrit values was recorded in LH (7.4%) followed by TH (7%) and KCH (5.9%) sheep, which roughly followed the trend of WC. Nonetheless, PCV criterion (based on natural infection) did not support LH sheep for being superior in natural resistance against *H. contortus* infection.

Total serum protein: There was a gradual decline in the values of TSP in all the three sheep breeds included in the study (Table III). Values of TSP remained different among three sheep breeds from 6th to 9th week post-exposure to contaminated pasture. The level of significance in difference increased with an increase in the level of infection i.e., FEC, WCs, etc. The maximum reduction in TSP values was recorded in TH (1.3 g/dL) followed by LH (1.1 g/dL) and KCH (0.9 g/dL) sheep.

Table I: Fecal egg counts in different sheep breeds naturally infected with *Haemonchus contortus*

Weeks (Post-infection)	Fecal Egg Counts (Mean±S.D.)*		
	Thalli	Lohi	Kachhi
3	16.6±30.46	3.3±12.91	13.3±35.18
4	33.3±44.98	26.6±53.00	23.3±37.16
5	83.3±64.55	76.6±59.36	86.6±71.88
6	130±99.64	126±163.51	130±153.29
7	133±101.18	103±118.72	116±123.44
8	196±121.69	180±165.61	190±116.80
9	286±182.70	203±142.00	236±121.69

*number of animals was 15

Table II: Packed cell volume (%) in different sheep breeds naturally infected with *Haemonchus contortus*

Weeks (Post-infection)	Packed cell volume (Mean±S.D.)*		
	Thalli	Lohi	Kachhi
2	27.4±1.26[a]	28.1±1.32[ab]	27.0±1.33[ac]
4	26.6±1.10[a]	27.8±0.94[b]	26.5±1.14[ac]
6	25.2±0.79[a]	26.4±0.93[b]	25.8±1.22[ab]
8	23.1±1.80[a]	24.4±0.67[b]	23.8±0.86[ac]
9	20.4±1.35[a]	20.7±1.40[a]	21.1±1.62[a]

Table III: Total serum proteins concentration (g/dl) in different sheep breeds naturally infected with *Haemonchus contortus*

Weeks (Post-infection)	Total serum proteins (Mean±S.D.)*		
	Thalli	Lohi	Kachhi
2	6.0±0.64[a]	6.3±0.55[a]	5.9±0.66[a]
4	5.7±0.51[a]	6.1±0.60[a]	5.8±0.61[a]
6	5.2±0.61[a]	5.6±0.63[b]	5.3±0.42[ab]
8	5.0±0.48[a]	5.5±0.58[b]	5.1±0.62[ac]
9	4.7±0.52[a]	5.2±0.49[b]	5.0±0.53[ab]

Table IV: Serum albumin concentration (g/dL) in different sheep breeds naturally infected with *Haemonchus contortus*

Weeks (Post-infection)	Serum albumin (Mean±S.D.)*		
	Thalli	Lohi	Kachhi
14	3.6±0.25[a]	3.5±0.14[a]	3.5±0.24[a]
28	3.4±0.18[a]	3.5±0.15[a]	3.4±0.17[a]
42	3.2±0.16[a]	3.4±0.13[b]	3.2±0.25[ac]
56	3.0±0.38[a]	3.2±0.59[a]	3.1±0.67[a]
63	2.8±0.50[a]	3.1±0.31[a]	2.9±0.29[a]

Table V: Live weight (kg) of different sheep breeds naturally infected with *Haemonchus contortus*

Weeks (Post-infection)	Live body weight (Mean±S.D.)*		
	Thalli	Lohi	Kachhi
2	25.4±1.92[a]	25.2±2.41[ab]	20.9±2.61[c]
4	26.0±2.58[a]	25.1±3.24[ab]	21.0±2.81[c]
6	25.0±2.62[a]	25.1±3.44[ab]	20.9±2.80[c]
8	25.1±2.57[a]	25.4±3.05[ab]	20.9±2.85[c]
9	25.9±3.08[a]	25.9±3.18[ab]	21.13±3.28[c]

*Number of animals was 15. Means marked with same letters in a row do not differ significantly ($P < 0.05$)

Serum albumin: Values of SA remained at par among three sheep breeds from 2nd to 9th week post-infection with an exception of 6th week (Table IV). Maximum reduction in SA was recorded in TH (0.8 g/dL) followed by KCH (0.6 g/dL) and LH (0.4 g/dL) sheep. LH sheep, therefore tolerated better the effects of *H. contortus* infection on SA values.

Live weight: Maximum weight gain (1.23 kg) was recorded in KCH sheep followed by LH (0.7 kg) and TH (0.5 kg) sheep (Table V). All the sheep breeds faced a negligible increase or decease in LW from 2nd to 6th week PI. From 6th to 9th week PI, however there was an increase in LW of all the sheep breeds included in the study.

DISCUSSION

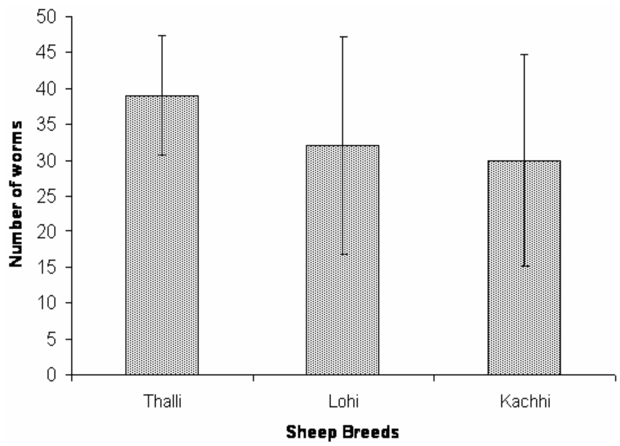
The results of natural pasture trial supported the findings of artificial trial infection (Saddiqi *et al.*, 2010) but level of infection remained paradoxically low that may be due to small number of experimental animals, low infection on newly selected pastures and development of resistant against *H. contortus* due to previous infection that has definitely low FECs as compared to primary (Bahirathan *et al.*, 1996; Gruner *et al.*, 2003) due to better immune response (Good *et al.*, 2006). Outcomes of present trial were in contrast to that of Aumont *et al.* (2003) who noted that the secondary infection amplified the breed effect on establishment of worms and accordingly FEC.

All the sheep breeds studied here showed almost same pattern of resistance status against haemonchosis at 6-8 months old age as was in previous infection (3-5 months) but difference among breeds with respect to haemonchosis remained negligible. LH breed showed low FECs as compared to KCH and TH but breeds differed insignificantly same as reported previously by many workers (Preston & Allonby, 1978; Li *et al.*, 2001; Chauhan *et al.*, 2003; Good *et al.*, 2006). FECs had been used extensively to evaluate natural resistance status as it is both a repeatable and heritable phenotypic trait.

Post-necropsy WC showed significant difference ($P < 0.05$) among breeds in contrast to FECs. TH breed was susceptible having relatively more WCs on the basis of this parasitological parameter. Variations in WCs have also been reported earlier between Menz and Horro lambs (Haile *et al.*, 2002), Suffolk and Texel lambs (Good *et al.*, 2006), Santa Ines and Suffolk and Ile de France (Amarante *et al.*, 2004), Canaria Hair and Canaria sheep (Gonzalez *et al.*, 2008). Variation in worm burden between breeds may arise from differences in grazing behavior, which could influence the intake of infective larvae (Good *et al.*, 2006).

PCV decreased from week 2 to 9 PI due to sucking of blood from abomasa by adult *H. contortus*. Results of haematocrit parameter remained analogous to WCs as PCV is negatively correlated with adult WC. Difference among breeds in PCV remained significant ($P < 0.05$) after the patency of infection from day 42 to 63 PI. PCV had been used by previous researchers for the evaluation of resistance and resilience of hosts in case of blood sucking parasites like *H. contortus* (Dargie & Allonby, 1975). Results of PCV

Fig. 1: Adult worm count at necropsy in different sheep breeds naturally infected with *Haemonchus contortus*



in our study resembles to the previous ones (Mugambi *et al.*, 1996; Bricarello *et al.*, 2004) but differed with Vanimisetti *et al.* (2004). Different reports exist about this parameter. Notter *et al.* (2003) found reduction in PCV percentage but stability in PCV was seen even in the presence of GIN infection (Hoffman, 1981).

With the increase of sudden infection from week 6 to 9 PI, TSP concentration decreased significantly within this period. Although KCH showed less reduction but mean values remained comparatively better in LH throughout the trial. TSP may decrease (Zajac *et al.*, 1990; Mugambi *et al.*, 1996) or not (Bricarello *et al.*, 2004; Gonzalez *et al.*, 2008) in the presence of GIN infection. SA concentration remained almost same (P > 0.05) except at day 42 PI (P < 0.05) due to sudden increase in FECs. SA had also been used as parameter in earlier trials (Mugambi *et al.*, 1996; Bricarello *et al.*, 2004).

LW has also its own significance in the judgment of resistance and susceptible status of sheep/goats as it was formerly implicated by different workers (Amarante *et al.*, 2004; Burke & Miller, 2004; Vanimisetti *et al.*, 2004; Mugambi *et al.*, 2005). All the breeds continue to grow but they can not gain normal weight due to haemonchosis. Breeds showed significant difference (P<0.001) at all the weighing times on the contrary to Vanimisetti *et al.* (2004). Usefulness of weight gain/loss criterion in the evaluation of different breeds of animals seems to depend on the uniformity or dis-similarity in the genetic performance potential and/or size of the animals.

Although mechanism of resistance against heamonchosis is not fully understood yet it is identified that 'resistant animals' have comparatively pronounced capability to withstand infection and expulsion of worms than 'susceptibles', which may be due to high level of IgE (Bricarello *et al.*, 2007), IgA (Gill *et al.*, 1994), mucosal mast cells (Gill *et al.*, 2000), globule leukocytes (Gamble & Zajac, 1992; Bricarello *et al.*, 2004) and eosinophils (Gill *et al.*, 2000; Amarante *et al.*, 2004).

It can be concluded that overall LH breed is moderately more resistant than KCH and TH. Its genetic potential against GINs could be explored more at large scale including more animals and genetic parameters.

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