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RESEARCH ARTICLE

Comparative Evaluation of Natural Resistance of Dera Din Panah and Nachi Goat Breeds Towards Artificial Infection with *Haemonchus contortus*

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ABSTRACT

Gastrointestinal nematodes (GINs) remain a major constraint associated with the profitable production of goats under grazing conditions. The present study was planned to determine the comparative susceptibility of Dera Din Panah (DDP) and Nachi breeds of goats towards artificial infection with *Haemonchus (H.) contortus*. To this end, a total of 24 goats of each breed were administered with third stage infective larvae of *H. contortus* through early and late infection protocols. The differences in faecal egg count (FEC), post necropsy worm count, rate of establishment of infection, packed cell volume (PCV), haemoglobin (Hb), eosinophils, total serum protein (TSP) and serum albumin (SA) were compared to check breed susceptibility to worms. Both breeds reflected significant ($P<0.05$) differences in aforementioned parameters at different time intervals post infection. However, Nachi breed showed a compromised response towards artificially infection with *H. contortus* as compared to DDP breed. Overall, higher FEC, higher number of adult worm recovery along with significant reduction in PCV and Hb depicted that Nachi is comparatively more susceptible to *H. contortus* infection as compared to DDP. In conclusion, difference in response towards *H. contortus* infection may formulate the base of selective breeding of resistant goat breed (DDP) in the area.

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INTRODUCTION

Goat (*Capra hircus*), the poor man's cow, is considered as integral part of various farming systems. Goat farming is playing a significant role in poverty reduction, particularly for non-agriculture community and resource poor farmers (Khan *et al*., 2008). Parasitism is one of the important issues all over the globe and reducing the productivity of animals (Farooqi *et al*., 2017; Luo *et al*., 2017). Among various parasites, gastrointestinal nematodes (GINs) have been recognised among threat to goat population by causing high morbidity and production losses around the globe (Nabi *et al*., 2014). Goats are exposed to different species of GINs during grazing e.g. *Haemonchus* (*H.*) *contortus, Trichostrongylus axei, Teladorsagia circumcincta* and *Marsahllagia marshali* etc. (Lamrioui *et al*., 2013; Nabi *et al*., 2014; Bibi *et al*., 2017). Various parasitic stages of these GINs nourish or feed on blood and cellular secretions of goats leading to direct and indirect losses. Among direct losses; anaemia, reduction in live weight, morbidity and mortality are of serious concern (Hoste *et al*., 2008). Indirect losses attributed to GINs are cost of repeated anthelmintic treatments and management. In Pakistan, losses of approximately 8800 million rupees are attributed to infection with GINs, particularly haemonchosis (Qamar *et al*., 2011).

Conventionally, control of GINs of small ruminants is achieved with broad spectrum anthelmintics (Khan *et al*., 2017; Mehmood *et al*., 2017; Zaman *et al*., 2017) and other husbandry practices. However, inappropriate use of anthelmintics has led to the development of anthelmintic resistance (AR). Furthermore, adulterated and ineffective drugs, problems of drugs residues in milk and meat of animals and unavailability of efficacious drugs are exacerbating the situation (Waller, 2006). Due to these emerging issues, scope of non-chemical strategies for the control of GINs are currently being investigated around the globe (Jackson *et al.,* 2007**)**. Exploring inherent resistance of goat breeds to parasites would reduce dependence on anthelmintic drugs. Scanty data are available to support idea of breed resistance to parasites, which demonstrates that specific goat breeds are resistant, tolerant or resilient and susceptible to GINs (Chiejina *et al*., 2010; Chauhan *et al*., 2014; Periasamy *et al*., 2014). Globally, different goat breeds from varying geography have been screened for their inherent resistance to GINs e.g. Barbari and Jamunapari goats (Chauhan *et al*., 2014), Black Iraqi goats (Al-Jebory and Al-Khayat, 2012) and Local Ardi goats (Al-Seaf and Khaled, 2012) and have been proved resistant towards some species of GINs including *H. contortus.*

Nature has blessed Pakistan with 37 breeds of indigenous goats distributed in five provinces (Khan *et al*., 2008). Up to now, in Pakistan, Beetal and Teddy goat breeds of upper Punjab province have been tested for their resistance towards *H. contortus* and results showed that Teddy breed is comparatively resistant to *H. contortus* as compared to Beetal breed (Shamim *et al*., 2016) while no reports are available on susceptibility or resistance status of common goat breeds of less developed region of southern Punjab. Thus, present study was carried out to determine the comparative susceptibility of Dera Din Panah (DDP) and Nachi breeds of goats towards artificial infection of *H. contortus* through some phenotypic markers.

MATERIALS AND METHODS

Female goats of less than 6 months in age (24 of each breed) were purchased from their native area and were brought to Department of Parasitology, University of Agriculture, Faisalabad, Pakistan (UAF). These goats were treated with anthelmintics to keep them worm free and acclimatized for four weeks. All goats were weighed, ear tagged and kept in separate pens. Goats were provided hay/concentrate feeding and *ad libitum* water till the end of experiment. All the experimental protocols were practiced as recommended by the Institutional Animal Care and Use Committee.

Experimental design: For the collection of 3rd stage infective larvae (L_3) of *H. contortus*, faeces of donor goats kept at UAF were subjected to coproculture (Zajac and Conboy, 2011). Larvae were recovered through Baermann's technique and stored at 4°C until used. Goats from each breed were divided into six random groups (n=4). Two infection protocols i.e. early and late infection, further divided into bolus and trickle forms of infection, were adopted to infect the treatment groups as described by Shamim *et al*. (2016). Briefly, in early infection protocol, first group of both breeds (D1, N1) was administered with a single bolus infection (18,000 L_3) on day zero while a dose of 6000 L_3 was given to trickle group of each breed on day zero followed by three successive doses of 2000 L_3 on every other day to get a total infection dose of 12000 L_3 (D2, N2). Similarly, late infection groups were infected with same protocols except for the late trickle group, which was administered with three successive doses of 2000 L_3 on every other day of second week to achieve a total infection dose of 18000 L_3. Two groups from each breed were kept as negative control, one for early treatment and other for late treatment. For each treatment group, faecal egg count (FEC), packed cell volume (PCV), haemoglobin (Hb), eosinophils, total serum protein (TSP) and serum albumin (SA) was determined on different days post infection (PI). At end of experiment i.e. 8th week, all goats were killed and number of *H. contortus* were counted from abomasum.

Experimental Protocols

Faecal egg count: Five-gram faeces were collected directly from the rectum of goats on weekly basis with complete sample description. Collected faecal samples were processed through qualitative and quantitative faecal examination protocols as described by Zajac and Conboy (2011).

Collection and processing of blood samples: Blood (10 mL) was collected directly from the jugular vein aseptically. For haematological examination and serum separation, blood was poured in vacutainer tubes having 0.5% EDTA and gel clot activator, respectively. Collection of sera was done in 2 mL Eppendorf tubes and kept at -20°C till further processing (Kiechle *et al*., 2010). Eosinophils, PCV and Hb were measured in automatic haematology analyser (Medonic Haematology Analyser), while SA and TSP were measured through spectrophotometric method (Benjamin, 1978).

Post-necropsy worm recovery: All the goats were killed and abomasa were collected for recovery of adult *H. contortus* by following protocol described by Siddiqi *et al*. (2010).

Statistical analyses: Differences between study goat breeds in terms of FEC, PCV, TSP, SA, live weight and post necropsy worm count was measured by analysis of variance and comparing their mean values. All the statistical analyses were carried out at 95% confidence interval and 5% precision level.

RESULTS

Different infection protocols showed non-significant (P>0.05) effect on any of the parasitological, biochemical or haematological parameter. Both study breeds of goats showed a significant (P<0.05) difference in study parameters from 4th to 8th weeks PI. A higher mean FEC (4512.5) was recorded in Nachi breed as compared to DDP goat (2687.5). Comparison of average FEC of both breeds has been presented in Fig. 1. Post-necropsy mean adult worm counts also corresponded to the values of FEC in study breeds of goat and found significantly (P<0.05) higher in Nachi breed (4250) than DDP (1125). Similarly, a marked difference was observed in rate of establishment of *H. contortus* infection in Nachi goat (23.61%) as compared to DDP breed (10.25%) and results were significantly associated (Fig. 1 and Fig. 2).

In both goat breeds, a decrease in PCV was observed on 4th, 6th and 8th weeks PI. However, observed differences in PCV values were significantly (P<0.05) in Nachi breed as compared to DDP breed (Fig. 3). There was a marked reduction in Hb levels during 2nd to 8th week PI. However, the reduction was highest at 8th week PI in infected groups of both breeds. The values of eosinophils count were

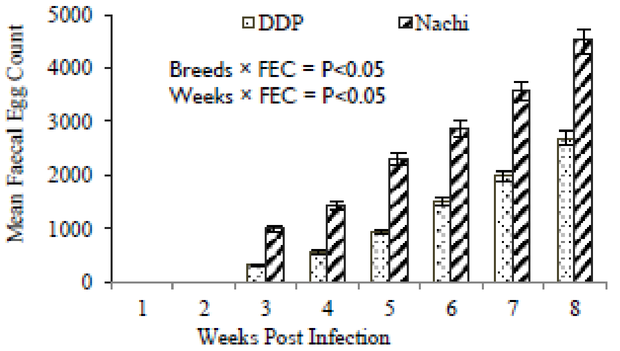

Fig. 1: Comparison of parasitological parameters (means±S.E) in Nachi and Dera Din Panah (DDP) goats infected with artificial infection of *Haemonchus contortus* at different time intervals post infection.

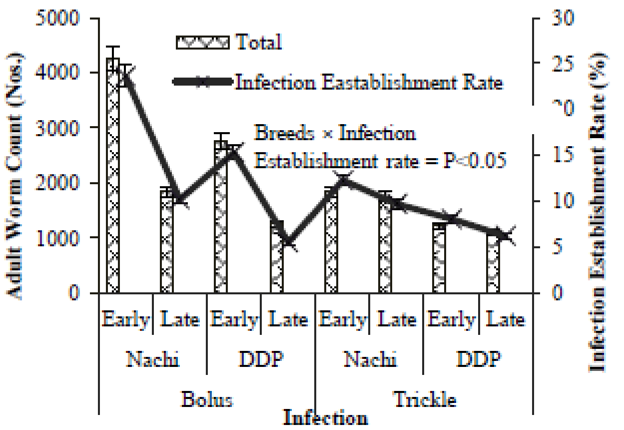

Fig. 2: Comparison of post necropsy mean worm counts in abomasa of Nachi and Dera Din Panah (DDP) goats after artificial infection of *Haemonchus contortus*.

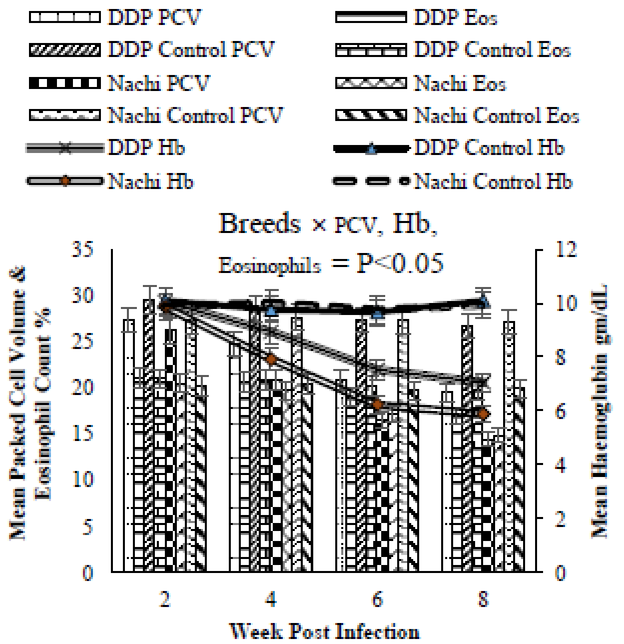

Fig. 3: Comparison of haematological parameters (means±S.E) in Nachi and Dera Din Panah (DDP) goats infected with *Haemonchus contortus.*

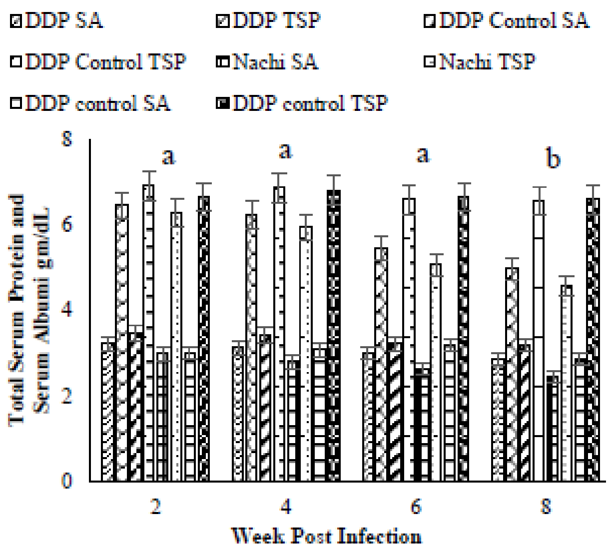

Fig. 4: Comparison of biochemical parameters (means±S.E) in Nachi and Dera Din Panah (DDP) goats infected with artificial infection of *Haemonchus contortus* at different time intervals. [a-b]Weeks with different superscript are significantly different (P<0.05).

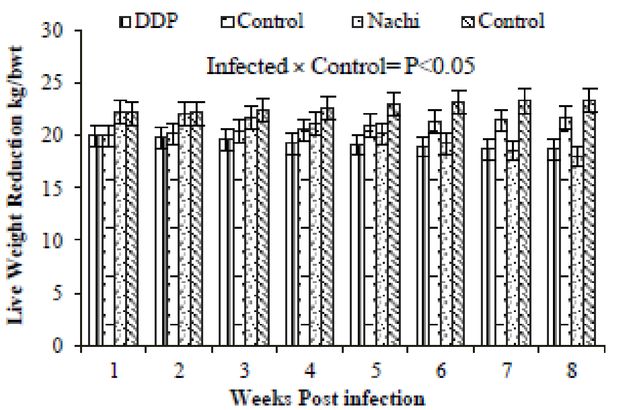

Fig. 5: Comparison of reduction in live weight (means±S.E) in Nachi and DDP goats infected with artificial infection of *Haemonchus contortus* at different time intervals.

significantly (P<0.05) different in Nachi goats as compared to DDP goats at different weeks PI. Eosinophils were higher at 4th week PI in infected groups of both goat breeds (Fig. 3). Similarly, a marked change in values of SA was observed in infected groups of both goat breeds at different time intervals PI (week 2nd to 8th). However, this change was significant (P<0.05) at 8th week PI in infected groups of both breeds (Fig. 4). The decline in SA was comparatively higher in Nachi goats (0.53 g/dl) as compared to DDP goats (0.38 g/dl). Both breeds of goats showed a gradual reduction in values of TSP from 2nd to 8th week PI. The reduction in values of TSP was comparatively higher in DDP (1.23 g/dl) goats than Nachi (0.6 g/dl). The live weight (LW) gain of infected groups of both breed was reduced from 2nd to 8th week PI. The drop in LW of Nachi goat (3.60 kg) was higher as compared to DDP goats (1.33 kg) during current experiment. Difference in LW reduction was different significantly (P<0.05) in Nachi and DDP goat breeds (Fig. 5).

DISCUSSION

Selection of genetically resistant breeds against GINs may serve as an alternate tool for their control (Shamim *et al*., 2016). Resistance from parasitological perspective is known as the host's ability to limit FEC during GINs infection. Resistant animals have a strong tendency to disturb the life cycle of a parasite. Hence, the establishment of infection is greatly affected in resistant host (Roeber *et al*., 2013). The rate of replication of a parasite inside its host is directly linked with the level of resistance of the host (Doeschl-Wilson *et al*., 2012). Resistance itself is a relative measure rather than an absolute one in terms of level of parasitic burden and time point at which host got infection (Periasamy *et al*., 2014). It is a proven fact that selective breeding of animals against various diseases is possible. Number of studies have reported variability in goat breed susceptibility to GINs e.g. Black Iraqi (Al-Jebory and Al-Khayat, 2012), West African Dwarf (Chiejina *et al*., 2010) and Cashmere goat breeds (McBean *et al*., 2016) have shown resistance towards GINs in general and *H. contortus* in particular. Phenotypic parameters like FEC, PCV, worm burden, infection establishment rate, TSP, SA level and live weight gain/reduction evaluated in the study breeds of present report have also been evaluated by other authors in small ruminants (Siddique *et al*., 2010; Baber *et al*., 2015; Shamim *et al*., 2016).

The most reliable and useful method to evaluate the presence of resistance among animals with same parasitic infection is FEC (McBean *et al*., 2016). Active parasitic infection is characterized by shedding of eggs of parasites in faeces of host (Gonzalez *et al*., 2008). There are some other factors, which may also be observed in the host for its inherent resistance against *H. contortus* including; lengthening of pre-patent period, low rate of establishment of infection and less worm burden during necropsy (Siddiqi *et al*., 2010; Shamim *et al*., 2016). Current results revealed higher FEC in Nachi goats as compared to those of DDP and similar trend has been seen in establishment of artificial infection. The present study revealed a significant difference between the number of parasites present and infection rate in DDP and Nachi. This may be attributed to the expulsion of larvae after infection which might be associated with the production of antibodies and release of histamine against *H. contortus*. This trend coincides with the findings of Miller *et al*. (1998). However, variations in FEC have been reported in different species of small ruminants (Gonzalez *et al*., 2008; Siddiqi *et al*., 2010). It has been reported that despite the significant differences in FEC values, Red Maasai and ¾ Red Maasai goats had significantly lesser worms as compared to Dorper and ¾ Dorper goats, respectively (Mugambi *et al*., 2005). Similarly, Shamim *et al*. (2016) reported that resistant host reflected lower worms than susceptible ones.

During natural and artificial infections with GINs, haematological parameters such as PCV, Hb and eosinophils are valuable. For blood feeding parasites like *H. contortus*, these parameters are very important because PCV and Hb may decrease due to their capacity to draw large quantity of blood (0.5 mL/day) by each adult parasite. During the current study, interaction between breed groups and weeks PI reflected significant differences in PCV and Hb values between two breeds. Similar kinds of results have been reported in various breeds of small ruminants including: Dorper, Sabi (Matika *et al*., 2003) and Canaria sheep (Gonzalez *et al*., 2008).

Biochemical parameters are among important tools to evaluate host resistance against infection. An important indicator of parasitic infections is hypoalbuminemia (Bordoloi *et al*., 2012). As far as concentrations of TSP and SA are concerned, non-significant differences were recorded in both goat breeds. However, these were significantly different on different weeks PI, which recommends variation in inherent resistance of the study breeds. It has already been reported that TSP of resistant breeds have less effected than susceptible (Gonzalez *et al*., 2008). Level of plasma proteins have been reported higher in resistant breeds than susceptible ones. Differences in albumin concentration between resistant and susceptible breeds of goats and sheep have been reported elsewhere (Bricarello *et al*., 2005).

Both Nachi and DDP are heavy goat breeds. DDP possesses greater growth rate than Nachi. The Nachi and DDP breed exhibits remarkable decrease in weight PI, which is more prominent in Nachi as compared to DDP. It has been reported that in a specific study period, the overall weight gain was very low in susceptible breeds of sheep (Mugambi *et al*., 2005). Weight gain and live body weight were insignificantly different between susceptible and resistance animals (Bricarello *et al*., 2005). In this study, the difference between weight gain in Nachi and DDP may be due to difference of genetic potential of the breeds. The criterion of weight loss and gain is considered a useful parameter which depends upon herd size and genetic potential of the animals (Notter *et al*., 2003).

Conclusions: Goat breed is a crucial factor that influences GIN infection especially the prevalence of *H. contortus* in goat population of South Punjab. Nachi breed showed compromised response to artificial infection with *H. contortus* as compared to DDP. Higher susceptibility of Nachi goats to *H. contortus* was seen based on phenotypic markers.

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Authors contribution: MI was involved in designing, execution of the experimental work/procedures, interpretation of data and manuscript writing. MNK was involved in procurement of experimental materials, selection of animals and collection and examination of samples. MSS was involved in conduction of experimentation, statistical analysis and drafting of manuscript. MS was involved in conduction of experimentation, statistical analysis and interpretation of data.

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