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Full Length Article

Phenotypic Marker Based Evaluation of Resistance to *Haemonchus contortus* in Teddy and Beetal Goat Breeds of Punjab, Pakistan

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Abstract

Goats (*Capra hircus*) are valued assets for resource poor farmers globally. Parasitic infections, especially *Haemonchus contortus* (Trichostrongylid), impact health and production of goats globally. The present study was therefore, intended to evaluate indigenous goat breeds (Teddy and Beetal) of Punjab, Pakistan for their resilience and resistance to experimental infection of *H. contortus*. Of 60 goats, 30 each of Teddy and Beetal breeds were further divided into six groups, each consisting of five goats. Four groups of each breed received challenged infection with 12000 and 18000 L_3 (third stage) larvae of *H. contortus* under two infection protocols *viz*; early and trickle and remaining two groups of each breed were kept as controls. Resilience and resistance of each breed was determined on the basis of comparative phenotypic markers like: faecal egg counts (FEC), packed cell volume (PCV), FAMACHA scores, live body weight (LW), total serum protein (TSP), serum albumin (SA) and worm count on 2nd, 4th, 6th and 8th week post artificial infection with *H. contortus*. Teddy breed showed significantly ($P<0.05$) better assessment rank based on phenotypic markers and tendency to resist in response to *H. contortus* infection as compared to Beetal. The sustainable goat farming should include Teddy as important entity to minimize the issue of anthelmintic resistance. Further, evaluation of genetic markers like: quantitative trait loci (QTLs), genes of major histocomplexcity, protein expression, immunoglobulins, histamines and specific interleukins are recommended for future studies, which can be helpful to explore genetic potential of goat breeds on the basis of their molecular makers.

Keywords: Goat; Beetal; Teddy; *Haemonchus contortus*; Resistance; Phenotypic markers

Introduction

Goats (*Capra hircus*) are source of earning for resource-poor farmers in agriculture-based countries like Pakistan. Goat rearing has been practiced mainly by people living in rural areas. Gastrointestinal (GI) nematodes in general and trichostrongyles in specific are one of the restrictions (Sharma *et al*., 2009), for goat production and health globally. Trichostrongyles e.g. *Haemonchus contortus* is among the major concerns in tropical and subtropical goat rearing areas (Di-cerbo *et al*., 2010; Paraud *et al*., 2010; Sindhu *et al.*, 2014). *H. contortus* is an abomasal nematode which imbibes approximately 0.05 mL blood/day causing anaemia, weight loss, unthriftiness and mortality during hyper-acute stage (Miller *et al*., 1998; Notter *et al*., 2003). A higher magnitude of economic losses has been found attributable to the *H. contortus* (Javed *et al*., 1992; Iqbal *et al*., 1993). Importance of chemotherapy cannot be flouted in spite of certain concerns like: anthelmintic resistance (Waller, 2006), which is very common in goats (Zajac and Gipson, 2000), higher cost of therapy, and drug residues in meat and milk of the treated animals (Kaufmann *et al*., 2011; Tsiboukis *et al*., 2013). These limitations compel researchers to ponder about sustainable alternate control of GI trichostrongyle nematodes including *H. contortus*.

In this context, evaluation of indigenous breeds for their response towards *H. contortus* (as an experimental model) is a rational approach. Selection of parasite resistant breeds is an alternate option for control of GI nematodes (Fakae *et al*., 2004). Further, it will be helpful in identifying genetic potential of breed response towards GI nematodes which can help overcome problem of anthelmintic resistance and having an overall better growth and performance (Baker *et al*., 2001). Reports are available which describe the variability of genetic potential of goat breeds towards *H. contortus* infection (Behnke *et al*., 2006; Chiejina *et al*., 2010; Alberti *et al*., 2012; Al-Jebory and Al-Khayat, 2012; Corley and Jarmon, 2012a; b). Similar reports are also available from some developing countries (Chiejina and Behnke, 2011). Pakistan is the third largest goat producing country with 66.6 million heads of goats and 37 well recognized goat breeds.

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However, a handful of investigations are available on determination of genetic potential of native goat breeds and their response towards *H. contortus* challenged infection. Therefore, present study was intended to evaluate two common indigenous goat breeds of Pakistan (Beetal and Teddy) to ascertain their response on the basis of some phenotypic markers against challenged infection with *H. contortus*.

Materials and Methods

Study Area

The present study was conducted from March to May 2014 at Small Animal Experimental Facility of the Department of Parasitology, Faculty of Veterinary Science, University of Agriculture Faisalabad, Punjab, Pakistan.

Experimental Goat Management

The present study was conducted for a period of 12 weeks including quarantine period on a total 60 goats comprised of 30 each of Teddy and Beetal breeds. The goats were aged 6 – 8 months with an average weight range of 18 to 21 kg, respectively. These goats were kept in a pre-prepared shed in separate pens. Experimental goats were offered hay and concentrate twice a day and *ad libitum* water till the end of the experiment. Prior to an experimental infection, all goats were acclimatized for a period of four weeks. All the goats were dewormed with albendazole and ivermectin according to the recommended doses to clear natural infection if existed. Faecal flotation, sedimentation and faecal culture techniques were performed to confirm complete elimination of earlier natural infection (Zajac and Conboy, 2011). All the experimental goats were weighed, ear tagged and kept in respective groups according to their phenotypic characters.

Procurement of *H. contortus* L_3 for Experimental Infection

For the procurement of third stage larvae (L_3), faecal sample were collected directly from rectum of naturally exposed goats to make a pool of positive samples for copro-culture following the procedure of Zajac and Conboy (2011). Third stage larvae (L_3) were isolated and collected through Baermann's technique and larvae were identified according to the morphological keys and stored at 4°C until next use.

Experimental Infection

Two each of early and late infection protocols i.e. single primary infective dose and trickle infective dose were followed to infect experimental goats to evaluate the response of experimental goats of each breeds (Beetal and Teddy groups hereafter abbreviated as B and T, respectively). The experimental goats were divided into 12 groups (six groups for each breed) with five goats in each group. Of six groups of each breed, four were infected with *H. contortus* L_3 stage and two were kept as controls. Each group of goats received infective L_3 stage of *H. contortus* in the following protocols: groups B1 and T1 received 18000 larvae as primary infection on day zero; whereas, groups B2 and T2 received a total of 12000 larvae in divided doses as 6000 L_3 on day zero and 2000 L_3 on subsequent day for three days (1, 2, 3). Groups B3 and T3 were kept as controls receiving no infection. Similarly, for late infection protocol, groups B4 and T4 received 18000 larvae as single dose on day zero; whereas, groups B5 and T5 received a total of 18000 larvae; 6000 on day zero of first week and 2000 on each subsequent day for three days (1, 2, 3) and 6000 on second week in divided dose rates of 2000 L_3 on each day for three days (1, 2, 3). An outline of the treatment protocol is given in Table 1.

Parasitological Procedures

Coprological examination: Faeces (five grams) were collected from experimental goats as per standard procedures of quantitative and qualitative examination and coproculture to determine the worm load, worm egg count and worm identification (Zajac and Conboy, 2011).

Blood Collection and Processing

Ten mL of blood samples were aseptically collected from goats using 18 gauge syringes directly from the jugular vein and transferred immediately to 0.5% ethylene diamine tetra acetate (EDTA) coated tubes/vacutainers for serum separation and haematology. Serum samples were stored at -20ºC till further processing (Kiechle *et al*., 2010). Haematological parameters were tested using the method described by Preston and Allonby (1978), Total protein and serum albumin levels/concentrations were determined using the spectrophotometric methods as described by Benjamin (1978).

Necropsy, Worm Recovery, Identification and Enumeration

For necropsy, worm recovery and enumeration, protocol of Saddiqi *et al*. (2010) was followed with some modifications. Briefly, at the end of 8^{th} week post infection (PI), all infected goats of both breed were slaughtered for procurement of adult *H. contortus* worm count (WC). After opening the abdominal cavity, abomasa were removed and opened along its 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.

Statistical Analysis

Statistical package (SAS, 2010) was used to analyse data to find goat breeds resistance/resilience towards *H. contortus* in term of FEC, PCV, body weight, serum albumin concentration, total protein concentration and FAMACHA score through analysis of variance. Results are presented in the form of mean and standard error of means. Significant factor level was considered at (P<0.05).

Results

Both Teddy and Beetal goat breeds showed variable responses through each phenotypic parameter at 2nd, 4th, 6th and 8th weeks PI. The mean values for FEC, PCV, FAMACHA score and live weight, were significantly (P<0.05) different within and between Teddy and Beetal goat breeds as shown in Fig. 1 (A through F); however, did not differ significantly for serum albumin and total serum protein values. It is noticed that variations of parameters between the breeds were much evident at 6th week PI. Teddy goats showed better resistance towards artificial infection with *H. contortus.* First batch of eggs of *H. contortus* appeared in faeces at the start of 3rd week PI and then the FEC increased gradually till the end of trial. In both breeds, there was no marked variation in PCV during first two weeks PI; however, haematocrit values started reducing from 4th week PI to the end of the trial. This reduction in PCV values were 5.8% and 7.7% in Teddy and Beetal, respectively. There were gradual and little reductions in total serum proteins and albumin levels which were more distinct in Beetal than Teddy. Vis-à-vis live weight, there was not much difference recorded in weight reduction in both breed of goats after *H. contortus* challenged infection; however, reduction in live weight was higher (1.2 kg) in Beetal than Teddy (0.87 kg). Qualitative estimation of anaemia through FAMACHA score card showed that Teddy goats have better tendency to cope with infection compared to Beetal. Enumeration of adult worms indicated higher total, dead and live adult *H. contortus* in the abomasa of Beetal than Teddy goats (Fig. 2), which showed better (P<0.05) establishment of infection in former (13%) than latter (10%). All the phenotypic markers were significantly varied (P<0.05) between the treatment and control groups in both breeds.

Discussion

Evaluation of goat breeds response towards GI nematode infection in general and *H. contortus* in specific provides an alternate way to reduce constraints of anthelmintic resistance (Waller, 2006). Number of studies (Baker *et al*., 2001; Mandonnet *et al*., 2001; Rout *et al*., 2011) have reported variability in goat breed susceptibility to GI infections. Some breeds of goats i.e., Black Iraqi (Al-Jebory and Al-Khayat, 2012), West African Dwarf (Behnke *et al*., 2003), Thai Native (Pralomkarn *et al*., 1997) and Cashmere goat breeds have shown resistance towards GI nematode in general and *H. contortus* in specific. Phenotypic parameters i.e. FEC, PCV, worm burden, infection establishment rate, total protein, serum albumin level and live weight gain/reduction evaluated in the study breeds of present report have also been reported elsewhere (Pralomkarn *et al*., 1997; Fakae *et al*., 2004; Baber *et al*., 2015). The phenotypic parameters have been used to assess the response of experimental animals against challenged infection with *H. contortus* to determine the comparative resistance/resilience to *H. contortus* in Teddy and Beetal goat breeds of Pakistan.

Ideally, resistant animals exhibit lower FEC and higher PCV; the former shows direct resistance/resilience in terms of extent of establishment of infection, while latter indirectly quantifies experimental animal capability to tolerate infection. Our results concluded that Teddy breed passed fewer eggs in faeces PI and maintain higher values for PCV, during course of infection. On the other hand, lower values of PCV represented by Beetal indicated its susceptibility towards infection as an important feature of susceptible breeds (Notter *et al*., 2003).

Live weight gain or reduction is also an indicator used for the estimation of resilience and resistance of small ruminants including sheep and goats by various researchers (Amarante *et al*., 2004; Burke and Miller, 2004; Vanimisetti *et al*., 2004; Mugambi *et al*., 2005). During the present study, both goat breeds grew continuously which shows that both have ability to endure heavy burden of infection, but this tolerance was higher in Teddy analogous to Beetal goat breed which may presumably be due to faster growth rate of Teddy. This variation in live weight reduction could also be due to genetics of an individual and/or breed (Rahman and Collins, 1990).

Anaemia and hypoproteinaemia have also been declared as known features of *H. contortus* infection (Rahman and Collins, 1990). Anaemia is the cardinal clinical sign of Haemonchosis due to blood loss as 0.5 mL blood drawn/day/worm. To assess the anaemic status, FAMACHA score card has been established, which has been proven very helpful tool in its diagnosis (Van Wyk and Bath, 2002), and evaluation of animal resistance and resilience (Bath *et al*., 2001), even in the field conditions. In the present investigation, Teddy goats showed an average FAMACHA score of 2, which indicated that this breed has better endurance ability towards anaemia which may lead to maintained higher PCV (Baber *et al*., 2015), than Beetal.

Variation in worm count at necropsy is also a valuable parameter to assess establishment of an infection. Our findings of higher susceptibility of Beetal breed than Teddy to an experimental *H. contortus* infection indicated through enumeration of worms has also been used for comparative breed susceptibility earlier (Amarante *et al*., 2004; Good *et al*., 2006; Gonzalez *et al*., 2008). Variation in worm burden between breeds may ascend from differences in physiology, genetics and phenotypic characteristics of breed as reported by Karlsson and Greeff (2012) and Ahmed *et al*. (2015).

Table 1: Layout plan of *in-vivo* evaluation of Teddy and Beetal goat breed resistance towards experimental infection of *H. contortus*

Weeks	Activity
1, 2, 3	Purchasing (n=60), Acclimatization, Blood and Faecal Sample Collection
3	Deworming (Levamisole and Albendazole)
1, 2, 3 PD*	Faecal Collection and Examination (EPG)
3rd PD	Artificial Infection (L3 stage of *H. contortus*)
1st PAI*	Faecal Collection and Examination (EPG)
2nd PAI	Faecal Collection and Examination (EPG) Blood Examination (Haematology & Biochemical Parameters)
3rd PAI	Faecal Collection and Examination (EPG)
4th PAI	Faecal Collection and Examination (EPG) Blood Examination (Haematology & Biochemical Parameters)
5th PAI	Faecal Collection and Examination (EPG)
6th PAI	Faecal Collection and Examination (EPG) Blood Examination (Haematology & Biochemical Parameters)
7th PAI	Faecal Collection and Examination (EPG)
8th PAI	SLAUGHTERING Faecal Collection and Examination (EPG, Adult worm count) Blood Examination (Haematology & Biochemical Parameters)

*PD = Post deworming; PAI = Post artificial infection

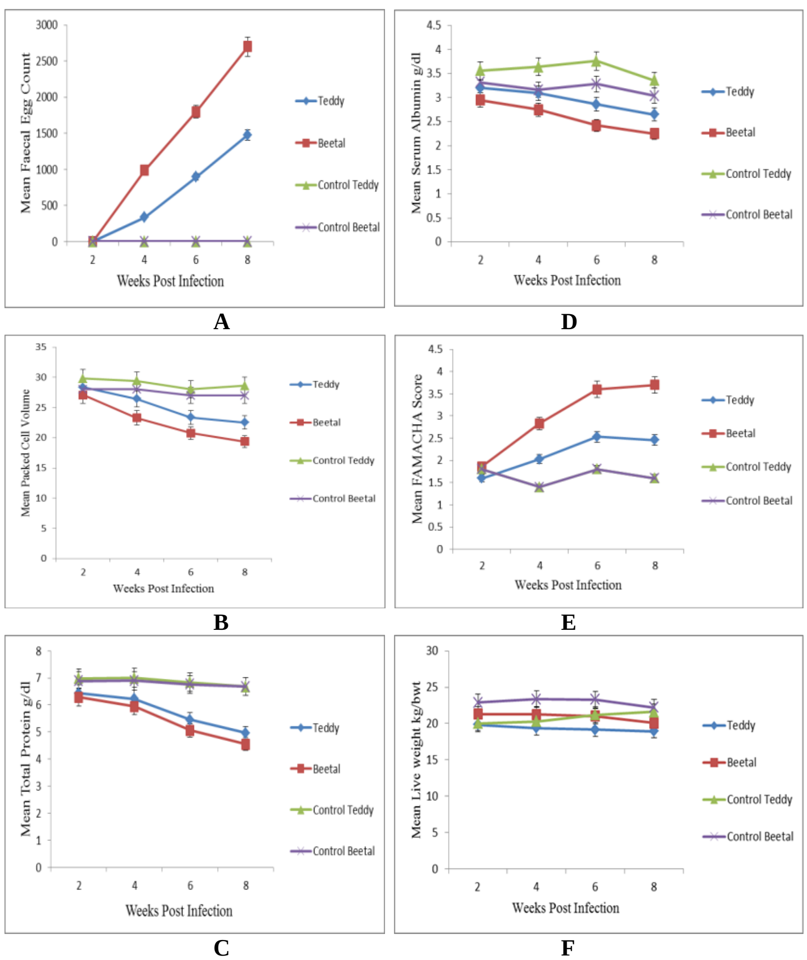

Fig. 1: Breed wise comparison of mean ± standard error of mean values of Teddy and Beetal goat breeds with their controls, A) EPG, B) PCV, C) TSP, D) SA, E) Famacha Score, F) LWR, against challenged infection of *H. contortus*

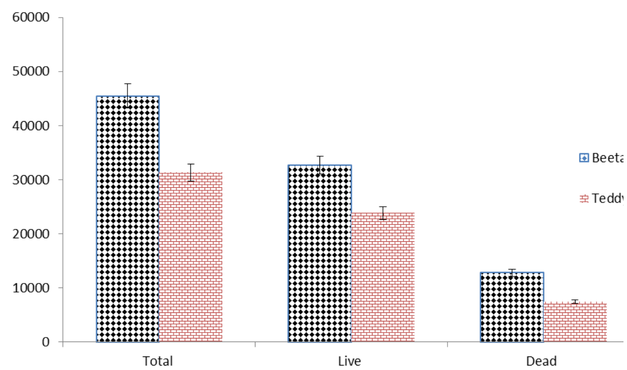

Fig. 2: Total, live and dead adult *Haemonchus contortus* recovered from abomasa of Teddy and Beetal goat breeds after artificial infection at Post mortem

Due to establishment of an infection from 4th week to 8th week PI values of total protein and serum albumin were altered in both breeds. There are reports that represent that variation existed between total protein and serum albumin concentration during infection period of GI nematode in general and *H. contortus* infection in particular (Bricarello *et al*., 2004; Mugambi *et al*., 2005; Gonzalez *et al*., 2008). Both total protein and serum albumin have been used as parameters for the evaluation of breed response to natural and artificial infection with *H. contortus* (Bricarello *et al*., 2004).

Mechanism of breed resistance towards GI in general and *H. contortus* in specific is not due to single entity, as it is correlated with activation and production of high level of immunoglobulins (Michael *et al*., 2004; Lee *et al*., 2011), mast cells, eosinophils (Alba-Hurtado and Muñoz-guzmán, 2013), high amines level (Rothwell *et al*., 1971), interleukins (Corley and Jarmon, 2012a; b), and influence of genetics of the breed. Resistant breeds show better expression of these substances than susceptible ones. Lower FEC, worm burden at necropsy and lower establishment rate confirm the resistance of Teddy over Beetal goats as reported for other breeds elsewhere (Pralomkarn *et al*., 1997). Further, association of genotype/ breeds with FEC and worm burden of goats has also been shown by Preston and Allonby (1978), Shavulimo *et al*. (1988), and Richard *et al*. (1990) for Galla, Saanen and East African goat breeds, respectively.

The results of present study have shown genotypic influences of the two goat breeds towards an artificial infection with *H. contortus*. It has been concluded that Teddy breed is found comparatively more resistant/resilient than Beetal breed on the basis of phenotypic parameters evaluated. Hence, breeding of Teddy can be encouraged by as backyard goats for the sustainable livelihood of the resource-poor farmers. However, potential of inherited resistance of Teddy goats against GI nematode in general and against *H. contortus* in particular could be further explored using higher number of animals and genetic investigations coupled with other phenotypic parameters to compliment the findings of present study. Utilization of modern biotechnology techniques such as microarray, single nucleotide polymorphism and proteomic analysis to identify variation in gene expression between susceptible and resistant goat breeds is also required.

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