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Variability in fecal egg count of *Haemonchus contortus* infection to native goat breeds of China and Bangladesh under natural grazing condition

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Abstract

Haemonchus contortus is a major gastrointestinal nematode affecting goat in China and Bangladesh like many countries in the world. The aim of this study was to evaluate the susceptibility of *Haemonchus contortus* infection in different goat breeds/populations in China and Bangladesh under the natural grazing condition. Fecal Egg of *Haemonchus contortus* was counted from 430 goats in both countries using McMaster's technique. Body weight, Pack Cell Volume and Hemoglobin value were measured for Bangladeshi goats. Statistical analysis was done after transforming data into $\log_{10}$ (n+1), where n is the number of egg per gram feces and analysis of variance was done by using Generalized Linear Model procedures of computer package of SAS. Prevalence of parasite and Fecal Egg Count varied significantly ($P<0.01$) in all six goat populations. The highest prevalence (88.89%) of *Haemonchus contortus* infection was found in Enshi Black and the highest parasitic load (527.74 ±78.13 epg) was found in Chinese hybrid goat population. Most of the individuals, except Chinese hybrid goats, had Fecal Egg Count less than 300 epg. Fecal Egg Count between sexes within breed of goat did not differ significantly ($P>0.05$). Black Bengal goat in the hilly region of Bangladesh was the least susceptible to *Haemonchus contortus* infection in term of Fecal Egg Count. Our study suggests that Chinese goat breeds were more susceptible to *Haemonchus contortus* infection as compared to Black Bengal goat of Bangladesh under natural grazing condition. Further studies on immune-genetics aspects of goats might be helpful to find out the actual causes of such differences and to develop disease resistant breed of goat.

Key words: Goat, *Haemonchus contortus*, fecal egg count, China, Bangladesh

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Introduction

Genetic resistance to gastrointestinal nematodes is a complex trait. Studies have revealed that the onset of *Haemonchus contortus* is often the result of the interaction between an individual animal's genetic makeup and the environment to which the animal is exposed (Gadahi *et al.*, 2016). Studies show that *Haemonchus contortus* exists in goat and sheep in 30 of 32 provinces of China (Yin *et al.* (2013). Black

Bengal goat is the only recognised goat breed in Bangladesh. Research done by Nozawa *et al.* (1988) and Afroz *et al.* (2010) reveals differences in morphology and genetic constitution among the known three populations of Black Bengal goat found in the Western part (BBW), the Central part (BBC), and the hilly regions in the Eastern part (BBH) of Bangladesh. In fact, *Haemonchus contortus* is the most frequent gastrointestinal nematode affecting the efficient production of goats in China and Bangladesh (He *et al.*, 2008; Hassan *et al*., 2011; Nahar *et al*., 2012; Yin *et al.,* 2013).

Studies have shown that if an animal has a genetic predisposition for acquiring a disease, then environmental conditions, including standard disease-prevention methods may only be partly effective in preventing the disease (Bharathi *et al.,* 2016). Till date, current methods to control the disease include vaccination, medication, sanitation, and isolation of animals from pathogens. These approaches, however, are seldom effective (Gilleard and Beech, 2007). Lack of effectiveness of some vaccines and increased attempts by consumers to change drug because of fears of contamination of human food sources underscore the need of more effective methods to combat *Haemonchus contortus* infection. A clear understanding of the disease and the goat's defence systems is required for alternative approaches to *Haemonchus contortus* infection control (Pena-Espinoza *et al.*, 2014). Von Samson-Himmelstjema and Blackhall (2005) reported that an often-overlooked alternative approach to standard disease control method would be selective breeding to increase disease resistant breeds in livestock. The feasibility of this approach has been experimentally demonstrated and breeding programs to select the animals for enhanced parasite resistance have been successfully established, especially for sheep (Bishop and Moris, 2007; McManus *et al*., 2014). Susceptible to nematode infections seems to be related to genetic factors and resistance may vary among breeds (Assenza *et al*., 2014). Studies have shown that measurement of heamatological parameters offers a means of detecting the presence of resistance within and among populations (Von Samson-Himmelstjema *et al.*, 2007). Kemper *et al.* (2011) showed the distribution and effects for faecal worm egg count in sheep, and the feasibility of using this marker to predict genetic merit for resistance was of crucial use. This study was, therefore, undertaken to evaluate genetically resistance or susceptibility to *Haemonchus contortus* infection between and within goat populations of China and Bangladesh under natural grazing condition that might be helpful to get basic information for innovation of the genetically resistant goat breed in future.

Materials and Methods

Ethics statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All animals used in this study were treated according to the guidelines for experimental animals established by the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

Experimental sites

In Bangladesh, experimental sites were two districts (Natore and Bandarban). GPS coordinates of two locations were N: $24^0$07.163′; E: $89^0$03.997′ and N: $24^0$47.523; E: $091^0$43.893 for Natore and Bandarban Hill district, respectively. Bandarban Hill district was hilly region and Natore was plain land of the country. In China, experimental animals were selected from southern China (Enshi, and Yichang) in Hubei province that represents the Southern part of China. GPS coordinates of two locations were N: $30^0$43’; E: $111^0$17’ and N: $30^0$17’; E: $109^0$29’ for Yichang and Enshi city respectively (Figure 1).

Animal selection and Sample collection

Present study were conducted in goat breeds of Yichang White (n=32), Nanjiang Yellow (n=56), Enshi Black (n=37), Chinese Crossbred goat (n=155) from

China and Black Bengal goat of Hilly region (BBH, n=37) and Black Bengal goat of western part (BBW, n=113) from Bangladesh (Figure 2).

Figure 1. A. Two regions of Natore and Bandarban district in Bangladesh; B: Two regions of Enshi city and Yichang city of Hubei province in China.

Figure 2. Different goat breeds or population of China and Bangladesh used in this study; (A) Nanjiang yellow, (B) Yichang white, (C) Enshi Black, (D) Chinese hybrid (Crossbred), (E) Black Bengal goat (Western region) and (F) Black Bengal goat (Hilly region).

Feacal samples were collected for Fecal Egg Counts (FEC) from each individual under natural grazing condition from May to July in 2014 and 2015 after the rainfall before deworming the animals. Age, Sex and Body weight were recorded. Blood samples were collected only from Bangladeshi goats for measurement of Pack Cell Volume (PCV) and Hemoglobin (Hb) value.

Faeces and hematological test

Fresh fecal sample (near to 5 gm) was collected by the two fingering method from each goat to estimate FEC. FEC were determined using saturated salt solution technique and were quantified using the modified McMaster's technique to determine fecal egg number and to identify different ova and oocyst of nematode species following Zajac and Conboy (2012) and expressed in eggs per gram (epg). The number of eggs per gram of faeces was calculated using the following formula:

Egg/gm = [no. of egg counted x (T/V)]/F

Where

T = Total volume of faeces/floatation solution mixture,

V = Volume of aliquot examined in slide,

F = grams of faeces used.

The sensitivity was 50 i.e. each egg represented 50 epg. Blood samples were collected for hematological assessment from jugular vein from Black Bengal goat in EDTA coated venoject tube and maintain proper cool chain before analysis. PCV was determined by the Micro hematocrit centrifuge method and result was expressed in percentage (%). Hemoglobin value was determined by the use of Sahli's acid haematin method and the result was expressed in g/dl (Table 3).

Statistical Analysis

Since the FEC data were not normally distributed, i.e. they were positively skewed, logarithmic transformation were applied before analysis. The descriptive statistics viz., arithmetic mean, variance, standard error, student t-test etc was done following the procedure described by Snedecor and Cochran (1980) after data were transformed into $\log_{10}$ (n+1), where n is the number of egg per gram feces. Analysis of variance was done by using Generalized Linear Model procedures of computer package of SAS (SAS Institute Inc. USA, 1998).

Results

A total of four hundred and thirty goats were examined for *Haemonchus contortus* infection by fecal egg count method. In China, 191 of 280 goats were found positive for *Haemonchus contortus* infection accounting for 68.21% infection rate. In Bangladesh, 87 of 150 goats were found positive for *Haemonchus contortus* infection representing 58.00% infection rate. The average FEC in Chinese and Bangladeshi goats was 402.86±46.60 epg and 158.00±31.30 epg respectively (Figure 3). The differences for prevalence and FEC of different goat populations in China and Bangladesh were highly significant ($P<0.01$) (Table 1).

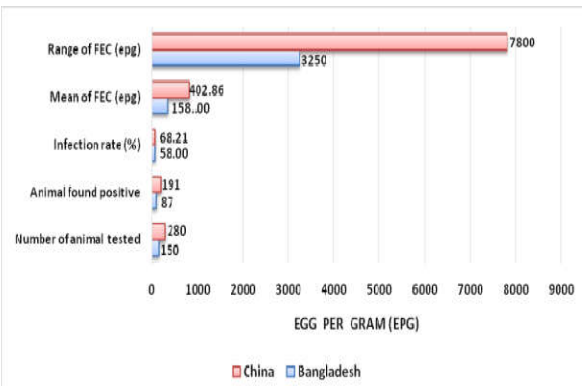

Figure 3. The relative infection of *Haemonchus contortus* in different goat breeds of China and Bangladesh under natural grazing condition

The highest (88.89%) and the lowest (51.79%) prevalence of parasite were found in ESB and NJY goat breed respectively. The highest parasitic load (527.74±78.13 epg) and the lowest parasitic load (201.56±47.62 epg) were, however, observed in Chinese Hybrid goat and YCW goat breed, respectively (Table 1). Table 1 revels that the prevalence of parasite was 72.79% and 53.10%, and the parasitic load was 112.16±17.37epg and 184.07±41.01epg in BBH and BBW population of Bangladesh, respectively. The differences for FEC among the breeds/populations were highly significant ($P<0.01$). The minimum and maximum FEC was 0 and 300 epg, 0 and 3250 epg, 0 and 1200 epg, 0 and 1800 epg, 0 and 2700 epg, 0 and 7800 epg for BBH, BBW, YCW, ESB, NJY and Chinese Hybrid breeds/population, respectively. However, more than 50% individuals in all the breeds/populations had FEC less than 300 epg. The percentage (%) of individual breed having FEC less than 300 epg were 100%, 86.78%, 76.78%, 72.72%, 66.66% and 58.33% for BBH, BBW, NJY, YCW, ESB and Chinese Hybrid goat (Figure 4).

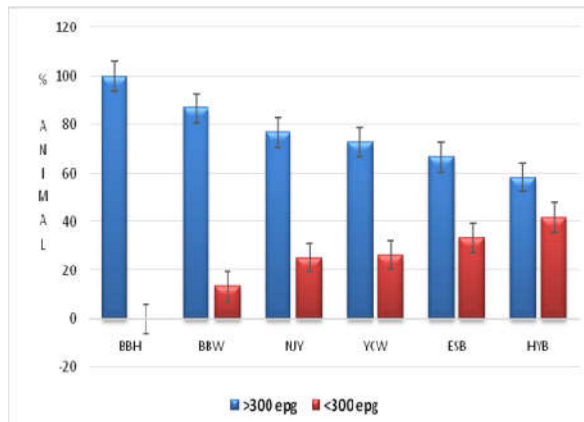

Figure 4. Degree of infection (%) of *Haemonchus contortus* in different goat breeds/ population between Bangladesh and China

The prevalence of *Haemonchus contortus* was more in female goats than male goats, only exception was BBH population where *Haemonchus contortus* was more prevalence in male goats than the female goats (Table 2). In BBH, NJY, ESB and Chinese Hybrid goats the parasite load in term of FEC was less in male goats than female goats. However, parasite load in term of FEC was more in male goats than female goats in BBW and YCW breed. The differences for FEC between sexes within breed were insignificant ($P>0.05$). Body weight and PCV% of goats increased with the advance of age and there was no significant difference

Table 1. Infection status of *Haemonchus contortus* in different breeds/populations of goat in Bangladesh and China

Breed Name	Total number of goat checked	Animal found positive for FEC	Prevalence (%)	Average FEC (epg)	Maximum FEC (epg)
BBH	37	27	72.97	112.16 ±17.37[a]	300
BBW	113	60	53.1	184.07± 41.01[b]	3250
YCW	33	22	66.67	201.56±47.62[c]	1200
NJY	56	29	51.79	220.54±58.41[d]	2700
ESB	36	32	88.89	331.94±68.11[e]	1800
Chines Hybrid	155	110	70.97	527.74±78.13[f]	7800
Level of significance				**	

a,b,c,d,e,f: Superscripts with different letters indicate difference among the data set in column; **Significant at P<0.01.

Table 2. Infection rate, Fecal Egg Count, and range of Fecal Egg Count of *Haemonchus contortus* in different sex of goat breeds in Bangladesh and China

Population	Sex	Animal found positive for FEC	Infection rate (%)	Fecal Egg Count (epg)	Range of FEC (epg)
BBH (37)	M (8)	7	87.5	87.50±32.39	0 to 300
	F (29)	20	68.97	118.97±20.37	0 to 300
BBW (113)	M (44)	21	47.73	246.59±83.91	0 to 3250
	F (69)	39	56.52	144.21±40.46	0 to 2050
YCW (33)	M (3)	2	66.67	250.00± 160.73	0 to 550
	F (30)	20	66.67	198.34± 50.66	0 to 1200
NJY (56)	M (0)	0	0	0	0
	F (56)	29	51.79	220.54±58.41	0 to 2700
ESB (36)	M (13)	11	84.62	265.43±68.73	0 to 850
	F (23)	21	91.3	369.57±99.59	0 to 1800
Chinese Hybrid(155)	M (27)	17	62.96	520.37±159.8	0 to 3600
	F (128)	91	71.09	529.3±88.66	0 to 7800

FEC: Fecal Egg Count; epg: Egg Per Gram; M: Male; F: Female; Figure in the parentheses indicates the number of goat examined

Table 3. Mean of Fecal Egg Count (FEC),Body Wight, Pack Cell Volume (PCV) and Hemoglobin (Hb) in Black Bengal goat for different sex and age groups exposed to natural *Haemonchus contortus* infection

Breed	Sex	Age	Number of Goat Studied	Mean ± Standard Error			
				FEC (epg)	Body Weight (kg)	PCV (%)	Hb (g/dl)
Black Bengal (150)	Male (52)	3m-5m	11	454.55 ±84.64	5.18 ±0.50	25.91 ±1.35	7.26 ±0.29
		6m –1yr	12	129.17 ±66.13	9.92 ±1.19	25.04 ±1.07	7.50 ± 0.35
		>1 yr	29	172.42 ±63.68	17.66 ±0.46	28.28 ±0.93	7.16 ± 0.14
	Female (98)	3m-5m	31	209.68 ±84.32	5.15 ±0.24	27.52 ±0.64	7.51 ± 0.23
		6m -1yr	40	103.75 ±17.67	11.35 ±0.67	27.66 ±0.55	7.90 ± 0.22
		>1 yr	27	101.85 ±31.41	20.81 ±0.79	27.00 ±0.79	7.77 ± 0.25

PCV: Pack Cell Volume; Hb: Hemoglobin; epg: Egg per gram; g/dl: Gram per deciliter; Kg: Kilogram; m: Month; yr, year; Figure in the parentheses indicates the number of goat examined in body weight and PCV in respect of sex in goats of Bangladesh (Table 3). However, Hb value remained almost same in respect of sex and age. Table 3 further reveals that the degree of infection of *Haemonchus contortus* deceased with the advance of age; FEC was, therefore, less in aged Black Bengal goats in Bangladesh.

Discussion

The occurrence of haemonchosis is influenced by many factors which comprises hosts, parasite and environment. Haemonchosis is primarily a disease of tropical and sub-tropical regions. In the present study, the data on *Haemonchus contortus* were collected from goats at subtropical environment in China and Bangladesh where goats were reared as scavenger in natural pasture. Moreover, samples were collected from May to August when humidity is relative high and favors heavy infection of *Haemonchus contortus* in goats as reported by many investigators in the world (Chiejina and Behnke, 2011; Yadev and Tandon, 1989; Fakae *et al.*, 1990; Raza *et al*., 2009).

The Chinese goat breeds had more parasite burden and infection than Bangladeshi goat breeds. These differences might be due to genetic factor like effect of breed as well as non-genetic factors like topography, climate and the animal management systems of two countries. Between breed variations in susceptibility to *Haemonchus contortus* is certainly a genetic factor and is evidence in variation of *Haemonchus contortus* among different populations and breeds of goat in Bangladesh and China. The higher parasitic load was observed in BBW population than BBH population in Bangladesh and YCW goat breed had the least parasitic load among four goat breeds in China. The variation in susceptibility to *Haemonchus contortus* expressed in term of FEC among breeds has been supported by previous studies of goat/sheep (Gray, 1995; Romjali *et al.*, 1996; Pralomkarn *et al.*, 1997; Costa *et al*., 2000; Chiejina and Behnke, 2011; McManus *et al*., 2014).

In Bangladesh, this is the first comparative study to evaluate the susceptibility availability to *Haemonchus contortus* infection in different populations of Black Bengal goat reared under natural constitution. Previous studies by Hassan *et al.* (2011), Akanda *et al*. (2012) and Nahar *et al*. (2012) were done either on single population. 30% prevalence of *Haemonchus contortus* was found by the investigation on the *Haemonchus contortus* infection in 20 Black Bengal goats of 18 months old reared in Govt. Goat Farm of Sylhet district (Akanda *et al.*, 2012). 41.79% prevalence of *Haemonchus contortus in* 317 Black Bengal goats of 4-60 months age reared under natural condition in Chittagong district located in the south eastern part of Bangladesh (Hassan *et al.*, 2011). However, Nahar *et al*. (2012) reported 57.8% prevalence of *Haemonchus contortus infection* in Black Bengal goat of Rajshahi district located in the western part of the country. In present study, the prevalence of parasite was higher than those reported by Hasan *et al.* (2011) and Nahar *et al*. (2012). This difference might be of seasonal effect. Hasan *et al*. (2011) collected samples in winter and summer season and Nahar *et al*. (2012) collected samples in all seasons of the year. Hassan *et al.* (2011) and Nahar *el al*. (2012) further reported that younger goats were more susceptible to *Haemonchus contortus than* the aged goats. Same trend was also observed for susceptibility of *Haemonchus contortus in* both populations of Black Bengal goat in the present study. Similar pattern of susceptibility to *Haemonchus contortus* have been reported in Chinese goats by He *et al.* (2008) and Ma *et al.* (2014). Ma *et al.* (2014) reported that 86% of examined adult goats from 6 months to 48 months of age were infected by different species of helminthes parasites while *Haemonchus contortus* has been found as the most prevailing nematode species with the prevalence of 81.8% during the summer season in Hunan province of China. He *et al*. (2008) revealed 49 species of different parasite worm detected from 506 individual goats by postmortem examination in which *Haemonchus contortus* were found as a dominant species of nematode whose infection rate was 40% among all those species in Hunan province of China. Elsewhere in Asia and Africa, Raza *et al.* (2009), Chiejina and Behnke (2011), Nabila *et al.* (2014) and Kumar *et al.* (2015) found the similar pattern of Haemonchus infection in goat. Kumar *et al*. (2015) studied on a total 290 Black Bengal goats (6 buck, 109 doe and 175 kids born from 11 sires) in West Bengal of India to evaluate the variability of resistance in Black Bengal goats that naturally infected with *Haemonchus contortus* with both genetic and non-genetic factors. Male kids had shown slightly higher resistance than female kids although it was not significant and resistance of kids was increased as age increases and kid population showed significantly different resistance status among the offspring resistant groups. Raza *et al.* (2009) found 31.10% prevalence of *Haemonchus contortus* in goats of Pakistan in which male had 29.91% prevalence and female had 31.90% prevalence. The similar seasonal, sexual and age variation in resistance to *Haemonchus contortus* of WAD goats of Africa has also been reported by Chiejina and Behnke, (2011). The findings of present study thus agree to the findings of Raza *et al.* (2009), Chiejina and Behnke (2011), and Kumar *et al.* (2015).

Packed cell volume and FEC are considered valuable for finding whether breeds have resistance against internal nematodes. The PCV is a measure of resilience by showing the animal's ability to endure against infestation, and FEC points out indirectly the resistant animal. Ideally, there should be low FEC and higher PCV in genetically resistant animals. These traits are key to evaluate the genetic resistance among breeds against nematode parasites. Since anaemia is the primary pathologic effect from infection with *Haemonchus contortus*, Hb value could be a valuable approach for selecting resistant breeds. However, this tool should be employed along with other tools (Bath *et al.,* 2001). PCV and Hb values along with FEC have been measured in judging resistance of sheep and goat to *Haemonchus contortus* by a number of investigators (Mandonnet *et al*., 2001; Babar *et al.*, 2015; Getachew *et al*., 2015). The same trend in PCV and Hb value along with FEC were observed in goat populations of Black Bengal goat in Bangladesh. Since blood samples of Chinese goats were not collected, so haematological parameters like PCV and Hb values could not be evaluated in Chinese goats.

Conclusion

The study indicates that Chinese goats were more susceptible to *Haemonchus contortus* infection as compared to Bangladeshi goats. Between and within population variation existed in breeds of goats in both countries. Younger goats were more susceptible to *Haemonchus* infection in both countries. Further studies on immune-genetics aspects of goats of two countries might be helpful to find out the actual causes of such differences and also help us to do association work to find out the disease resistant breed of goats.

Conflict in list of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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