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FACTORS AFFECTING VARIABILITY OF RESISTANCE IN GAROLE SHEEP NATURALLY INFECTED WITH *HAEMONCHUS CONTORTUS*

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ABSTRACT: Resistance status against natural infection to *Haemonchus contortus* as well as influence of season, sex, body weight, and haemoglobin type on resistance levels were evaluated in 309 numbers of Garole sheep. In adult Garole sheep, egg per gram(EPG) of faeces for *Haemonchus contortus* was varied from 300 to 1600, but overall EPG in Garole have been recorded as 829.96 ± 20.60. The effects of season, sex, and body weight and haemoglobin type on EPG were all found to be highly significant ($P < 0.01$). EPG count was highest during monsoon (986.27 ± 28.26), followed by summer (832.88 ± 28.26) and lowest during winter (670.74 ± 28.26) which indicated the existence of a seasonal variation of EPG. Rams had higher EPG (954.32 ± 57.93) than ewes (705.60 ± 45.79) which reflected that males appeared to be more susceptible to *Haemonchus contortus* infection compared to females. Animals with lower body weight (upto 10 kg) showed higher EPG (1017.20 ± 54.82), then the infection level decreased as body weight increased (886.79 ± 56.23 for 10 kg to 12 kg and 737.18 ± 50.29 for 12 kg to 14 kg) and lowest EPG was recorded in animals with above 14 kg body weight (678.68 ± 54.49). This study reveals Hb-BB type animals had higher EPG count (983.81 ± 18.22) in comparison to Hb-AB type animals (676.12 ± 33.96) indicating that Haemoglobin-A locus has some relation with resistance. From our study it can be concluded that resistant level of Garole sheep against *Haemonchus contortus* is influenced by some intrinsic factors like sex, body weight, and haemoglobin type of the sheep and also by extrinsic factor like season.

Key words: Garole sheep, *Haemonchus contortus*, EPG, Resistance, Haemoglobin type.

INTRODUCTION

Gastro-intestinal parasite infections are the major constraint to the sheep industry causing production losses, increased costs of management and treatment, and even mortality in severe cases (Barger and Cox 1984, Larsen *et al.* 1995). Most of the genera (of parasites) found in ruminants stimulate a relatively effective level of immunity in most animals after several months on pasture. This immunity

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significantly reduces the number of worms that become established in the grazing animals, the exception to this is *Haemonchus contortus* in sheep. In sheep, lambs below the age of 8 months do not develop significant protective immune responses to infection or immunization (Urquhart *et al.* 1996). This leads to prolonged susceptibility to reinfection is a major reason in lambs and therefore *H. contortus* remains the most economically important GI nematodes in sheep.

Faecal egg counts have been shown to have moderate (0.61) to high (0.91) correlation with nematode burdens (Baker *et al.* 1991, Stear *et al.* 1995, Bisset *et al.* 1996) and in essence might be considered a direct measure of infection. The most effective and only, practical way to estimate potential pasture contamination is from FEC and in one sense, this is also direct selection.

Several factors affect resistance to internal parasites, including intrinsic factors like sex of the sheep and extrinsic factors like season (Stear and Wakelin, 1998). Haemoglobin type also found to be associated with resistance level to *H. contortus* (Allonby and Urquhart 1976, Altaif and Dargie 1978, Preston and Allonby 1979, Kassai *et al.* 1990).

The main habitat of Garole sheep is the saline belt of Sunderban Delta of South 24 Parganas, some parts of North 24 Parganas and some saline zone of Purba Medinipur district of West Bengal, India. The climate is hot and humid. These sheep are indigenous and is popular for its biannual lambing with multiple births and their ability to graze on aquatic weeds and grasses standing even in knee-deep water for hours together (Kar and Prasad 1992).

The research in connection to variability of resistance against natural infection of H*aemonchus contortus* in Garole sheep is very limited although infections exist and are widespread. Realizing the importance of the subject, a research programme has been taken up in Garole sheep to study the variability of resistance against natural infection of *Haemonchus contortus* in Garole sheep as well as to evaluate the relationship of response to natural infection of *H. contortus* with haemoglobin genotypes / phenovariants.

MATERIALS AND METHODS

Site of experiment and animal :

The research work was carried out with 103 nos. of adult Garole sheep (*Ovis aries)* of which 45 were male and 58 were female, maintained in a farm under National Agricultural Technology project on 'Animal Genetic Resource Biodiversity' of West Bengal University of Animal and Fishery Sciences, Mohanpur Campus, Nadia, West Bengal, India. The farm is situated at $88^0$32¢E longitude and $22^0$56¢N latitude with an altitude of 9.75 m above mean sea level and having sub-tropical humid climate. On the basis of temperature, relative humidity and rainfall, the year has been divided into three seasons *viz.* winter (from November to February), summer (from March to June) and monsoon (from July to October). During the experiment period temperature varied within the range of 7.27ºC to 39.98ºC and relative humidity varied from 47.18% to 97.69%.

General management practice:

All the experimental animals were allowed for grazing on natural pasture from 8 A.M. to 3 P.M. in winter months. During summer and monsoon animals were grazed in two shifts *viz.* from 7 A.M. to 10.30 A.M. and 2.30 P.M. to 5.30 P.M. The pasture comprised mainly of doob grass (*Cynodon dactylon*) with other grasses and leaves *viz.* goose grass (*Eleusine indica*), horse purseane (*Trianthema monogyna*) and anjan grass (*Cenchrus ciliaris*). In addition to grazing all the animals were offered about 50g concentrates after returning from grazing in the evening. Adlibitum water was provided to all the animals in all seasons. All the ewes and rams were dewormed for 3 times in a year with Fenbendazole or Albendazole and Rafoxanide (7.5 mg to 10 mg/kg body weight orally) in three different seasons (summer, monsoon and winter). Animals were given dips from time to time to protect them from ectoparasites.

Collection of faecal sample and estimation of parasitic load:

Since sheep were maintained in a common natural pasture throughout the year, it was assumed that they got equal dose of *Haemonchus* infection from the grazing field. The faecal samples from each animal were collected rectally after one week of deworming and examined for *Haemonchus contortus* eggs. During each season two faecal samples were collected from each animal at five days interval and accordingly eggs per gram of faces (EPG) were determined following Stoll's Dilution Method as described by Soulsby (1982).

Collection of blood samples and determination of hemoglobin pheno-variants:

About 5 ml of fresh blood was aseptically collected from each animal by puncturing jugular vein with 0.5 ml of anticoagulant (Sodium citrate) and accordingly hemoglobin polymorphism was conducted following the Starch gel electrophoresis method described by Smithies (1955).

Grouping of animals according to body weight:

Body weights of adult sheep (rams and ewes) were taken in the morning with the help of spring balance (SALTER), which ranges from 8.5 kg to 18.0 kg. Thus sheep were grouped according to their body weight (Table 1).

Table 1: Body weight groups of adult sheep.

Body weight of sheep	Body weight group
Up to 10 Kgs	Group-I
< 10 Kgs to 12 Kgs	Group-II
< 12 Kgs to 14 Kgs	Group-III
< 14 Kgs	Group-IV

Statistical Analysis:

All the data were then subjected to Least-Squares analysis (1966) for studying the effects of season, sex and body weight and haemoglobin type on EPG of adult sheep. The model used for analysis was-

$$Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + e_{ijklm}$$

Where Y_{ijkl} is the observation on the m^{th} individual in i^{th} season of j^{th} sex of k^{th} body weight group and l^{th} haemoglobin type.

μ = General effect (Overall mean common to all observations);

A_i = Effect of the i^{th} season (i=1,2,3);

B_j = Effect of the j^{th} sex (j=1,2);

C_k = Effect of the k^{th} body weight group (k=1,2,3,4);

D_l = Effect of the l^{th} haemoglobin type (l=1,2) and

e_{ijkl} = random error assumed to be normally distributed with zero mean and variance,$ó^2_e$.

Duncan's Multiple Range Test (Kramer

1957) was performed to examine the significant differences between means whenever the analysis of variance showed significant differences for different factors.

RESULTS AND DISCUSSION

The resistance status was evaluated by measuring the level of *Haemonchus contortus* infections through eggs per gram in faeces (EPG). In the present study the EPG count in the Garole sheep was varied from 300 to 1600.

In Garole sheep the overall EPG for *Haemonchus contortus* have been recorded as 829.96 ± 20.60 (Table 2). The effect of season, sex, body weight and haemoglobin type was all found to be highly significant ($P < 0.01$) on EPG.

SEASON: EPG count was highest during monsoon (986.27 ± 28.26), followed by summer (832.88 ± 28.26) and lowest during winter (670.74 ± 28.26). This indicates the existence of a seasonal variation in patterns of FEC (Table 2 & Fig.1). The findings of Tembely *et al.* (1998) and Doligalska *et al.* (1997) were in agreement with the results obtained in this study with respect to the natural infection on pasture. Seasonal variation in FEC is most likely to be dependent on variation in availability of infective larvae on pastures. Two peaks in FEC can be seen: one during early summer due to development of over-wintered larvae; and another in monsoon depending on weather condition (Gregory *et al.* 1940; Whittier *et al.*

Table 2 : Least Squares means of EPG in adult sheep.

Effects	**Number**	**EPG** (Mean ± SE)
Overall	309	829.96 ± 20.60
Season:		
Summer	103	832.87 ± 28.26^{b}
Monsoon	103	986.27 ± 28.26^{a}
Winter	103	670.74 ± 28.26^{c}
Sex:		
Male	135	954.32 ± 57.93^{a}
Female	174	705.60 ± 45.79^{b}
Body weight group:		
Group-I	87	1017.20 ± 54.82^{a}
Group-II	78	886.79 ± 56.23^{b}
Group-III	30	737.18 ± 50.30^{b}
Group-IV	114	678.68 ± 54.49^{b}
Haemoglobin type:		
Hb-BB	252	983.80 ± 18.22^{a}
Hb-AB	57	676.12 ± 33.96^{b}

Means under a particular effect in a column having different superscripts differed significantly.

1997). During monsoon animals were exposed to higher infection levels on pasture, owing to favourable conditions for larval survival leading to increase in FEC. But in winter the phenomenon is just reverse leading to lowest FEC.

SEX: Rams had higher EPG (954.32 ± 57.93) than ewes (705.60 ± 45.79) as presented in Table 2 & Fig. 2. Male sheep appeared to be more susceptible to *H. contortus* infections compared to female sheep. Courtney *et al.* (1985a) Barger (1993), Woolaston and Piper (1996) also found that females were more resistance to natural as well as experimental infection with *T. colubriformis* and *H. contortus*. Females had lower FEC than males after puberty, although there were no differences before puberty. Our results with adult sheep are thus consistent with earlier reports. These differences may be due to a stimulatory effect of estrogens on immune responses and that androgens may actually have an opposite effect (Woolaston and Piper 1996).

BODY WEIGHT: Animals with lower body weight *i.e.* group I animals showed higher EPG (107.20 ± 54.82), then the infection level decreases as body weight increases (*i.e.* for group II EPG was 886.79 ± 56.23 and for group-III EPG was 737.18 ± 50.29) and lowest EPG was recorded in group IV animals (678.68 ± 54.49). The appreciable differences amongst the groups as encountered in the present study (Table 2 & Fig. 3) may be attributed due to differences in immuno-competent resistance status against natural infection of *H. contortus*.

Hb Type : It has also been observed that Hb-BB type animals (Fig. 4) showed higher EPG count (983.81 ± 18.22) in comparison to Hb-AB type animals (676.12 ± 33.96) as presented in Table 2 & Fig. 5. The reports of Allonby and Urquhart (1976), Altaif and Dargie (1978), Preston and Allonby (1979b) were in agreement with the results of our study. They reported that animals with haemoglobin type-AA (HbAA) were more resistant than Hb-AB, which again more resistant than Hb-BB to infection with *H. contortus* and its effects. This study also revealed that haemoglobin-A locus was related with resistance and/ susceptibility

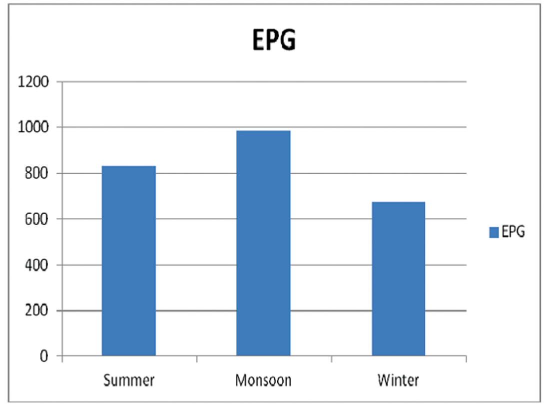

Fig. 1: EPG of adult Garole sheep in different seasons.

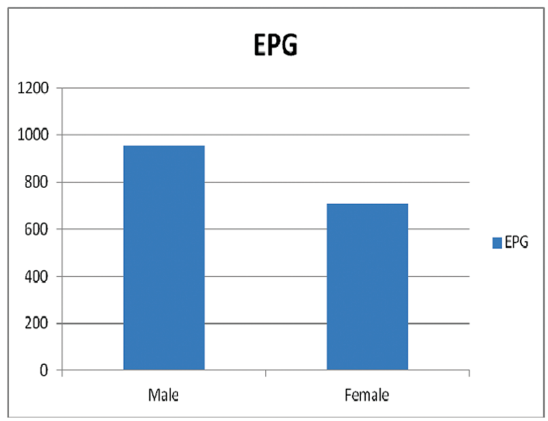

Fig. 2: EPG of adult Garole sheep in different sexes.

CONCLUSION

The variability of resistance to natural infection of *H. contortus* in Garole sheep was studied for some intrinsic factor like age, sex and body weight of sheep and extrinsic factor like season. This study recorded significant variation ($p < 0.01$) of response to natural infection for all these intrinsic and extrinsic factors. Lowest faecal egg count (EPG) was observed in winter and highest in monsoon. Male was found to be more susceptible to

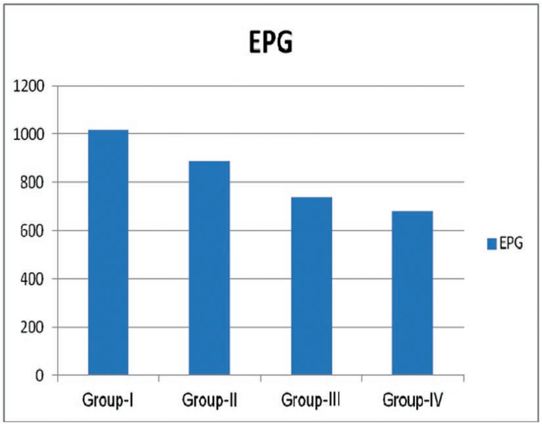

Fig. 3: EPG of adult Garole sheep in different body weight groups.

infection rather than female. In adult sheep the higher body weight sheep possessed significantly higher resistance than the lower body weight group. Haemoglobin AB type animals showed significantly (P<0.01) better resistance than the Haemoglobin-BB type animals. On the basis of the results obtained and discussion it is concluded that variability of resistance to infection with *H. contortus* is existed in Garole sheep and there is ample scope for increasing the resistance through selective breeding / assortive mating. During selection inclusion of haemoglobin type as an indicator trait may be helpful to achieve the goal early.

Further investigation on resistance status and variation in the artificial infection as well as identification of specific marker gene for resistance to *H. contortus* may be carried out as these are beyond the purview of the present study. This may be give us a clear picture about the genetic basis of resistance to *H. contortus* infection in Garole sheep, which may be helpful to develop a resistant population.

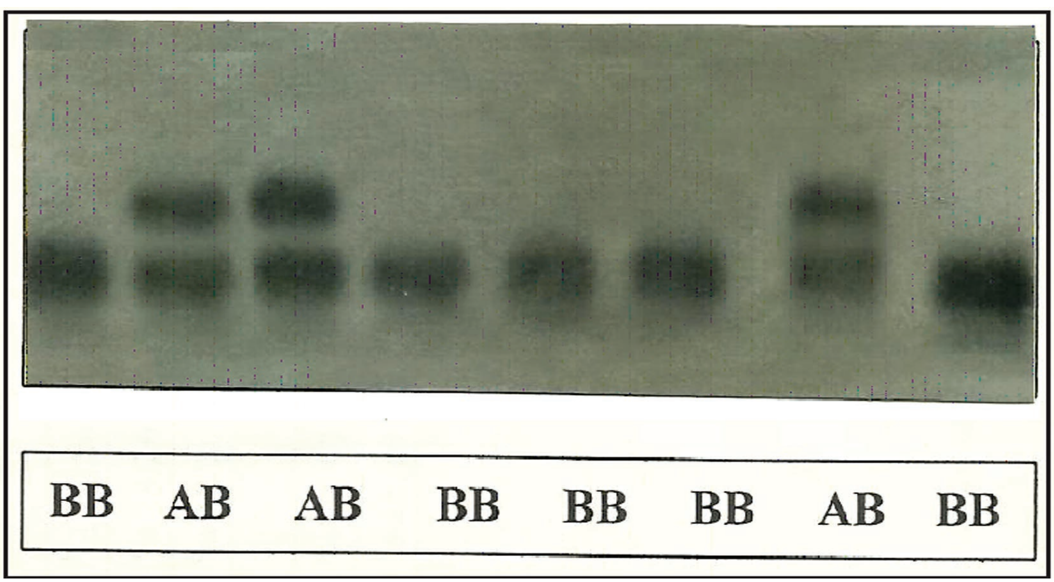

Fig. 4: Starchgel electrophoresis pattern of different haemoglobin.

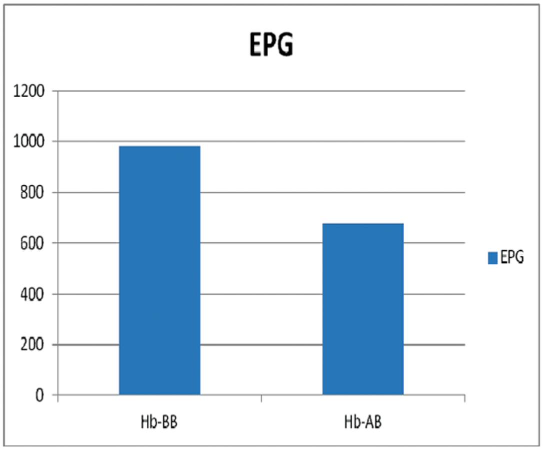

Fig. 5: EPG of adult Garole sheep in different Haemoglobin phenovariants.

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