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VARIABILITY OF RESISTANCE TO NATURAL *HAEMONCHUS CONTORTUS* INFECTION *VIS-A-VIS* HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN GAROLE SHEEP

Manoranjan Roy*[1], P.K.Senapati[2], Sukanta Roy[3] and Debraj Nandi[4]

ABSTRACT: A total 103 numbers of Garole sheep was evaluated to know the variability in resistant status against natural infection to *Haemonchus contortus* as well as variability in level of haematological and biochemical parameters. A significantly (P < 0.01) lower level of EPG, neutrophil and serum alkaline phosphatase enzyme, but significantly (P < 0.01) higher level of haemoglobin, packed cell volume, total leukocyte count, lymphocyte, serum total protein, serum albumin and serum globulin were recorded in resistant animals as compared to less susceptible and highly susceptible animals. Further a highly significant (P < 0.01) and negative relationship were observed for EPG with haemoglobin, PCV, TLC, lymphocyte, STP, SA and SG. Whereas a highly significant (P < 0.01) and positive relationship were observed for EPG with neutrophil count and SAP enzyme level. Significant variations as observed in the present study can be attributed to differences in FEC among the animals and these haematological as well as biochemical parameters can be used as a predictive marker for selection of *H. contortus* resistant Garole sheep in field condition in combating nematode infection which in turn results in efficient production.

Key words: Garole sheep, *Haemonchus contortus*, EPG, resistance, Haematological and biochemical parameters, Correlation.

INTRODUCTION

Modern day anthelmintics have been an important tool to control the parasitic infection in small animals, a number of factors have arisen recently that illustrate that dependency on drugs as a sole mean of parasite control is a serious mistake. In small ruminants, parasites have developed resistance against all classes of anthelmintics currently employed, and resistance to multiple classes of drugs by the same parasite in commonplace (Waller and Prichard 1986, Prichard 1990, Windon 1991, Waller 1994). A second concern about this dependence on anthelmintics is the growing insistence of consumers that their food and environment be free of chemical residues

[1]Assistant Professor, Department of Animal Genetics & Breeding, West Bengal University of Animal & Fishery Sciences, 37, Kshudiram Bose Sarani, Kolkata – 700037, West Bengal, India.
[2]*Professor, Department of Animal Genetics & Breeding, West Bengal University of Animal & Fishery Sciences, 37, Kshudiram Bose Sarani, Kolkata – 700037, West Bengal, India.*
[3]*Assistant Director, ARD (Administration), Bankura, West Bengal, India.*
[4]*Veterinary Officer, ABAHC, Matha Bagmundi Dev. Block, Purulia, West Bengal, India.*
[*]*Corresponding author*

(Herd *et al.* 1993). The growing numbers of 'organic' producers find repeated anthelmintic use to be an unacceptable means of parasite control. The third factor affecting current control programmes is the movement towards more intensive grazing programmes for livestock due to urban development and use of lands for recreational or conservational purposes. This move towards more intense use of pastures will necessitate more frequent use of anthelmintics, further increasing the chances for selection of anthelmintic resistance in the parasite populations. Goal of effective nematode control rogrammes is to protect animals from production losses and one such approach is to use genetically superior stock as an adjunct to current methods of control. There are some direct and indirect means to help make those selections. (Gasbarre and Miller 2000). Indicator traits usually reflect host response to infection, which includes some haematological and biochemical parameters (Khan *et al.* 1988, Ahmad and Ansari 1989, Mottelib *et al.* 1992, Yadav *et al.* 1993, Garcia-Baratute *et al.* 1994, Ghulam *et al.* 1995, Stear *et al.* 1995, Hayat *et al.* 1996, Yadav *et al.* 1997, Moskwa *et al.* 1998, Singh and Yadav 1998, Amarante *et al.* 1999, Hooda *et al.* 1999, Dhanalakshmi *et al.* 2002, Vanimisetti 2003) that can be used as potential tool for selecting sheep with increased resistance.

But research in this line is limited particularly in Garole sheep although *H. contortus* infections exist and is widespread in West Bengal. Realizing the importance of the subject, a research programme has been taken up in Garole sheep to study the variability of resistance to natural *H. contortus* infection and the effect of resistance status on haematological and biochemical parameters in order to obtain markers for selection of *H. contortus* resistance sheep if any.

MATERIALS AND METHODS

Site of experiment and animal

This research work was carried out on 103 numbers of Garole sheep (*Ovis aries)* of which 45 were Ram and 58 were Ewe (with age ranges from 12 - 40 months and body weight ranges from 9.5 - 16.8 kg), maintained at West Bengal University of Animal and Fishery Sciences, Mohanpur Campus, Nadia, West Bengal, India under National Agricultural Technology project on "Animal Genetic Resource Biodiversity" during the period of December'2001 to December'2004. The farm is situated at $88^0$32¢E longitudes and $22^0$56¢N latitudes and which is 9.75 m above mean sea level with hot-humid climate. During the experiment period temperatures vary from 7.27ºC to 39.98ºC and relative humidity varied from 47.18% to 97.69%.

General management

All the sheep under study were allowed for grazing on natural pasture from 8 A.M. to 3 P.M. in winter months (November to February). During summer (March to June) and monsoon (July to October) 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. In addition to grazing all the animals were offered about 50g of concentrates after returning from grazing in the evening. Adlibitum water was provided to all the animals in all seasons.

The animals were housed in groups during night in sheds. They were provided with floor space as per B.I.S. Routine vaccinations against endemic diseases of this location were carried out. All the sheep were clinically checked

Table1 : Groups of sheep according to parasitic load.

Status	Parasitic load (EPG)
Resistant	Up to 600
Less susceptible	Above 600 - up to 1200
Highly susceptible	Above 1200

routinely and de-wormed regularly with Fenbendazole or Albendazole and Rafoxanide @7.5 mg to 10 mg/kg body weight orally for 3 times in a year. Animal's sheds were cleaned every day. Animals were given dips from time to time to protect them from ectoparasites.

Experimental design

Since sheep were maintained in a common natural pasture throughout the year, it was assumed that they got equal dose of *H. contortus* infection from the grazing field. Sheep were grouped into resistant, less susceptible and highly susceptible to *H. contortus* on the basis of eggs per gram of faces (EPG). Animals with EPG up to 600, above 600 up to 1200 and above 1200 were considered as resistant, less susceptible and highly susceptible respectively. All the sheep were evaluated for their parasitic load, haematological and biochemical parameters.

Collection of faecal sample for parasitic load

After one week of deworming faecal samples were collected rectally from each animal and examined in three different seasons (summer, monsoon and winter). During each season two faecal samples were collected from each animal at five days interval for evaluation of *H. contortus* eggs. Eggs per gram of faces were determined following Stoll's Dilution Method as described by Soulsby (1982).

Collection of blood

From each animal about 10 ml of blood was collected by jugular vein puncture and out of this 5 ml was mixed with anticoagulant *i.e.* 4.0 mg Ethylene diamine tetra acetic acid (EDTA) disodium salt for haematological examinations and rest 5 ml kept as such for collection of serum and collected serum was

Table 2: Least squares means of Haematological parameters in adult Garole sheep.

Effects	No.	EPG	Haemoglobin (g/dl)	PCV (%)	TLC (10^3/mm^3)	Lymphocyte (%) (%)	Neutrophil
		Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Overall	103	936.884±12.939	8.577±0.065	24.071±0.151	6.508±0.063	50.564±0.320	42.160±0.244
Significant level		**	**	**	**	**	**
Resistant	23	$595.652±24.389^{c}$	$9.481±0.123^{a}$	$26.195±0.285^{a}$	$6.957±0.119^{a}$	$53.788±0.604^{a}$	$39.754±0.459^{b}$
Less susceptible	60	$885.000±15.100^{b}$	$8.423±0.076^{b}$	$23.552±0.177^{b}$	$6.269±0.074^{b}$	$49.547±0.374^{b}$	$42.932±0.284^{a}$
Highly susceptible	20	$1330.000±26.154^{a}$	$7.828±0.132^{c}$	$22.467±0.306^{c}$	$6.300±0.128^{b}$	$48.359±0.647^{b}$	$43.794±0.492^{a}$

** Significant at the 0.01 level.

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

Table 3: Phenotypic correlations of EPG with haematological parameters in adult Garole sheep.

Parameters	Phenotypic correlations
EPG with haemoglobin	-0.679**
EPG with PCV	-0.692**
EPG with TLC	-0.545**
EPG with lymphocyte count	-0.533**
EPG with neutrophil count	0.520**

** Significant at the 0.01 level.

kept in deep freeze at - 20^{0}C temperature till evaluation of biochemical parameters. During blood collection (from jugular vein) three thin blood smears were also made from each animal.

Parameters studied

Differential leukocyte count (%) was performed as per standard method of Schalm *et al.* (1975). Haemoglobin level (g/dl) was determined by Cynmethaemoglobin Method (Cannan 1958), packed cell volume (%) was determined as per standard method of Schalm *et al*. (1975), total leukocyte count was enumerated by haemocytometer as per standard method of Schalm *et al*. (1975) in terms of thousands per cubic millimetre (10^3/mm^3).

Serum total protein and albumin in each sample was determined by Modified Biuret and Dumas Methods of Reinhold (1953) in a Photoelectric colorimeter using a yellow green filter supplied along with Diagnostic Reagent kit for the *in vitro* determination of total protein and albumin in serum (Manufactured by Span diagnostics Ltd.). The globulin fraction in the serum samples was calculated by subtraction of serum albumin from total serum protein. The total serum protein, serum albumin and serum globulin values were expressed as g/dl of serum. Serum alkaline phosphatase enzyme activity was measured using 4-amino antipyrine by the method described by Kind & Kings (1954) using calorimeter and were expressed as KA Units.

Statistical Analysis

Least-Squares analysis (Harvey 1966) was performed for the variability of resistant as well as the effects of resistance status on haematological and biochemical parameters. The model used for analysis was:

$Y_{ij} = ì + A_i + e_{ij}$

Where Y_{ij} is the observation on the i^{th} individual in j^{th} Resistance group.

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

A_i = Effect of the i^{th} resistance group (i=1,2,3) and

e_{ij} = 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 is significant.

Phenotypic correlations (r_p) of EPG with different haematological and biochemical parameters were estimated as per the method described by Becker (1967).

RESULTS AND DISCUSSION

Parasitic load

The mean egg per gram of faeces (EPG) in adult Garole sheep was recorded as 936.884±12.939 (Table 2). Resistant animals showed significantly ($P < 0.01$) lower EPG count (595.652±24.389), followed by less

Table 4: Least squares means of Biochemical parameters in adult Garole sheep.

Effects	Number	EPG	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alkaline phosphatase (KA Unit)
		Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Overall	103	936.884±12.939	7.494±0.078	2.747±0.030	4.747±0.055	14.494±0.195
Significant level		**	**	**	**	**
Resistant	23	595.652±24.389^{c}	8.207±0.147^{a}	2.952±0.057^{a}	5.257±0.104^{a}	12.180±0.368^{c}
Less susceptible	60	885.000±15.100^{b}	7.298±0.091^{b}	2.637±0.035^{b}	4.662±0.065^{b}	14.177±0.228^{b}
Highly susceptible	20	1330.000±26.154^{a}	6.977±0.157^{b}	2.654±0.061^{b}	4.323±0.112^{c}	17.126±0.395^{a}

** Significant at the 0.01 level.
Means under a particular effect in a column having different superscripts differed significantly.

Table 5: Phenotypic correlations of EPG with biochemical parameters in adult Garole sheep.

Parameters	Phenotypic correlations
EPG with Serum total protein level	-0.574**
EPG with Serum albumin level	-0.255**
EPG with Serum globulin level	-0.552**
EPG with Serum alkaline phosphatase level	0.643**

**Significant at the 0.01 level.

susceptible (885.000±15.100) and highest EPG count was recorded in susceptible animals (1330.000±26.154).

Haematological parameters

The mean haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), lymphocyte count (LC) and neutrophil count (NC) in Garole sheep were recorded as 8.577±0.065 g/dl, 24.071±0.151 %, 6.508±0.063 x10^3/mm^3, 50.564±0.320 % and 42.160±0.244 % respectively (Table 2). The Hb, PCV and TLC and LC vary highly significantly ($P < 0.01$) with the resistance status of animals but in reverse directions. Resistant animals had higher values compared to less susceptible and highly susceptible animals (Hb: 9.481±0.123 vs 8.423±0.076 & 7.828±0.132 g/dl), (PCV: 26.195±0.285 vs 23.552±0.177 & 22.467±0.306 %), (TLC: 6.957±0.119 vs 6.269±0.074 & 6.300±0.128 x10^3/mm^3) and (LC: 53.788±0.604 vs 49.547±0.374 & 48.359±0.647 %). But in contrast to this neutrophil count was recorded lowest in resistant animals (39.754±0.459 %), followed by less susceptible (42.932±0.284 %) and

highest value (43.794±0.492 %) was recorded in highly susceptible animals. In our study the differences of TLC and NC in between less susceptible and highly susceptible animals were found to be non-significant.

Lower Hb, PCV, TLC and LC in highly infected sheep are expected because blood is sucked by these parasites from the intestinal wall for their growth, maturation and maintenance as well as for phagocytosis. Results of our experiment may be compared with the findings of Ahmad and Ansari (1989), Dhanalakshmi *et al.* (2002), Yadav *et al.* (1993, 1997), Ghulam *et al.* (1995) and Hooda *et al.* (1999) who reported significantly lower level of haemoglobin in highly infected sheep; Albers *et al*. (1987) and Vanimisetti (2003) who reported lower PCV in animals with higher FEC; Ghulam *et al.* (1995), Mottelib *et al.* (1992), Dhanalakshmi (2002) and Moskwa *et al.* (1998) who reported significantly higher level of lymphocyte count in resistant sheep (with lower FEC) than susceptible (with higher FEC). Although no reference is available for TLC and NC, but the present contention may be explained on the fact that resistant sheep had lower *H. contortus* infection as measured by lower EPG.

Phenotypic correlation of EPG with haematological parameters

Phenotypic correlations estimated between EPG with Hb, PCV, TLC and LC were observed to be negative and highly significant (-0.679, -0.692, -0.545 and -0.533 respectively) in Garole sheep (Table 3).Whereas the phenotypic association between EPG and NC were positive and highly significant (0.520).

Biochemical parameters

The overall least square mean of total serum protein (TSP), serum albumin (SA), serum globulin (SG) and serum alkaline phosphatase enzyme (SAP) level in Garole sheep were recorded as 7.494±0.078 g/dl, 2.747±0.030 g/dl, 4.747±0.055 g/dl and 14.494±0.195 KA Unit respectively (Table 4). Resistance status had produced a highly significant ($P < 0.01$) effect on TSP, SA, SG and SAP levels. Higher levels of TSP, SA and SG were recorded in resistant animals in comparison to less susceptible and highly susceptible animals (TSP: 8.207±0.147 vs 7.298±0.091 & 6.977±0.157 g/dl ; SA: 2.952±0.057 vs 2.637±0.035 & 2.654±0.061 g/dl and SG: 5.257±0.104 vs 4.662±0.065 & 4.323±0.112 g/dl). But the differences of TSP and SA levels in between less susceptible and highly susceptible animals were found to be non -significant. Conversely highly susceptible animals had highest level of SAP (17.126±0.395 KA Unit), resistant animals possessed the lowest value (12.180±0.368 KA Unit) and less susceptible animals obsessed intermediate level (14.177±0.228 KA Unit).

This variation in TSP, SA and SG content among the different resistance groups may be due to variability of level of *H. contortus* infection. Resistant animal had lowest EPG which imposed them to produce higher level of STP, SA and SG level, whereas the susceptible animals are not capable to produce optimum amounts. This results may be compared to the findings of Ahmad and Ansari (1989) who reported significant higher level of SAP enzyme in heavily infected sheep with higher FEC than those of resistance once (with lower FEC).

Phenotypic correlation of EPG with biochemical parameters

Phenotypic correlations estimated between EPG with STP, SA and SG were observed to be negative and highly significant (-0.574, -0.255 and -0.552 respectively) in Garole sheep (Table 5). Where as the phenotypic association between EPG and SAP enzyme was positive and highly significant (0.643).

CONCLUSION

The variability of resistance to natural infection of *H. contortus* vis a vis some haematological and biochemical parameters was studied in Garole sheep which revealed a significant variation ($p< 0.01$) of parasitic load in Garole give a way to understand the resistance status. The variation in resistance leads a significant variation in haematological and biochemical parameters in Garole sheep. Resistant animals showed significantly ($P<0.01$) lower EPG than the susceptible animals. Resistant animals also possessed significantly higher level of haemoglobin, packed cell volume, total leukocyte count, lymphocyte count; serum total protein, serum albumin and globulin than susceptible animals. On the other hand susceptible animals possessed significantly higher level of neutrophil and serum alkaline phosphatase enzyme than resistant animals. Our study also quantitatively demonstrated that protein metabolism was significantly affected by *H. contortus* infection in sheep. Lower serum concentrations of protein in highly susceptible animals may be due to malabsorption and / or loss of protein from gut having high *H. contortus* infection.

On the basis of the results it is concluded that variability of resistance to infection with *H. contortus* is existed in Garole sheep. During selection inclusion of haemoglobin, packed cell volume, total leukocyte count, lymphocyte count, neutrophil count, serum total protein, serum albumin, serum globulin and serum alkaline phosphatase enzyme as indicator traits 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 which might give us a clear picture about the genetic basis of resistance to *H. contortus* infection in Garole sheep and helpful to develop a resistant population.

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