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Genetic Studies on Faecal Egg Counts and Packed Cell Volume Following Natural *Haemonchus contortus* Infection and Their Relationships with Liveweight in Muzaffarnagari Sheep

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ABSTRACT : A total of 437 animals, comprising lambs aged between 3 and 12 months and adults of either sex of Muzaffarnagari sheep maintained at the Central Institute for Research on Goats, Makhdoom, Farah, Mathura, India were screened to assess the prevalence of *Haemonchus contortus* infection following natural infection and to identify the various factors affecting faecal egg count (FEC) and packed cell volume (PCV) of ewes and their genetic control. The relationships between FEC, PCV and body weight were also estimated. The prevalence rate for *H. contortus* infection in the flock under study was 15.7% indicating much lower occurrence of worm infection in lambs up to one year of age. On the other hand, a large proportion i.e., 67.7% of sheep was refractive to natural *H. contortus* infection. The random effect of sire significantly contributed ($p<0.01$) variation in log-transformed FEC (LFEC) of ewes. The season of birth had a significant ($p<0.01$) effect on LFEC of ewes. The lactating ewes had significantly ($p<0.01$) higher faecal egg counts compared to dry and pregnant ewes. The linear regression effects of the age of ewes on LFEC of animals were significant ($p<0.01$) in the present study. The heritabilities of LFEC, PCV and body weights of ewes during the course of infection were moderate to high in magnitude and ranged from 0.24 to 0.47. The LFEC of ewes was significantly ($p<0.05$) and negatively correlated with PCV at both genetic and phenotypic level. The genetic and phenotypic relationships between LFEC and body weights of ewes were -0.26 and -0.06 for this breed. The genetic correlation of PCV and body weight of ewes was positive and high (0.58) and statistically significant ($p<0.05$) but it was negatively correlated (-0.01) with body weight at the phenotypic level. (**Key Words :** Genetic Parameters, *Haemonchus contortus*, Faecal Egg Count, Packed Cell Volume, Sheep)

INTRODUCTION

Gastrointestinal nematode infections are a major constraint to the sheep industry, which causes production losses, increase cost of management and treatment, and even mortality in severe cases (Larsen et al., 1995). *Haemonchus contortus* infection of gastrointestinal tract is one of the major causes of wastages and decrease productivity through loss of blood and plasma protein in gastrointestinal tract. There have been several reports of endoparasites becoming resistant to most of the available classes of anthelmintic (Gopal et al., 1999). Progressive insufficient of chemo prophylactic therapy to control helminthiasis in sheep has a major contributing factor in stimulating research in to the development of alternative means of internal parasitic control. Over the past several years, evidence has emerged that suggests a genetic basis for resistance to gastrointestinal nematodes in sheep. Numerous reports are available on genetic variations within sheep breeds (Bishop et al., 1996; Morris et al., 1997; Woolaston and Windon, 2001) in resistance to infection by gastrointestinal helminthes. However, no evidence of any genetic differences of resistance to *H. contortus* infection in grazing Muzaffarnagari sheep, an important mutton breed in India, is available so far. Hence, the present study was undertaken with an aim to assess the prevalence of Haemonchus contortus infection following natural infection in lambs and ewes and to identify various factors affecting the faecal egg count (FEC) and packed cell volume (PCV) of ewes and further to estimate the genetic parameters for these traits along with their relationships with live weight of ewes.

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MATERIALS AND METHODS

Experimental flock and design

Data were collected from both lambs and adults of Muzaffarnagari sheep, maintained at the Central Institute for Research on Goats, Makhdoom, Farah, Mathura, India. A total of 437 animals which includes 90 lambs and 347 adults were considered for the study. The 90 lambs in this experimental design were the descendants of 85 ewes and 10 rams and all the lambs were born in a particular season. The number of lambs distributed in different age categories viz. 3-6 month, 6-9 month and 9-12 months were 26, 36 and 28, respectively. Out of 347 adult sheep, there were 270 ewes and 77 rams. These ewes were the offspring of 22 sires over the 4 years of period. The flock of this breed was established since 1976. The flock had been closed to outside breeding for several years and as a result, mil inbreeding develops in the flock (Mandal et al., 2005). In brief, the Muzaffarnagari, one of the heaviest and largest mutton breeds in the north-western region of India, is known for its faster growth rate and high feed conversion efficiency. Generally, the animals were housed separately according to their ages, sex, physiological status and health status and were maintained in semi-intensive system of management with 6 h of grazing and were supplemented with 250-300 g of concentrate ration according to their age and physiological status. Weaning of lambs is normally practiced at the age of 3 months. Controlled breeding was practiced in the flock. Two breeding seasons namely (1) May-June and (2) October-November, was practiced with lambing in October-November and March-April months of the year. Lambs and ewes were dewormed in the month of July-August. However, detailed husbandry practices of the breed were described by Mandal et al. (2000). Susceptibility to *Haemonchus contortus* infection following natural infection was evaluated in lambs and grazing ewes. The faecal samples were collected from rectum of different groups of animals and faecal egg counting was conducted using the modified McMaster technique (MAFF, 1977; Preston and Allonby, 1978) with each egg counted representing 200 eggs per gram of faeces. Faecal cultures were prepared from pooled faecal samples taken from a random 5% sample of the animals to define the species composition of the nematodes infecting the flock. Blood samples were taken on the day of faecal sampling to measure haematocrit value, which is packed cell volume (PCV). The blood was taken from the jugular vein of animals in standard 5ml vacutainer tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) containing heparin with the help of sterile syringe. Packed cell volume (%) was estimated by the micro-haematocrit centrifuge method (Preston and Allonby, 1978). The body weight of all animals was measured on the same day following collection of blood and faecal samples. The weights of the animals were recorded using a spring balance (Salter Pvt. Ltd., New Delhi, India) of 100 kg capacity.

Data and statistical analyses

Observations on 437 animals on faecal egg count were considered to study the prevalence of nematode infection in the flock. Data on FEC, PCV and body weights of ewes were used for subsequent analyses. The ewes were grouped on the basis of their physiological status viz. dry, lactating and pregnant. The birth status of animals was classified as single and twin. Faecal egg counts were not normally distributed and because of skewed distribution, a set of logarithms transformation was applied to FEC and the resulting transformed variables were tested for normality before analysis. The most appropriate transformation $\log_e$ (FEC+100) was used to correct for heterogeneity of variance and to produce approximately normally distributed data. All raw data of faecal egg count was transformed by $\log_e$ (FEC+100). The results were back transformed by taking anti-logarithms of the least-squares means (LSM), subtracting 100 and presented as geometric means (GFEC).

The data were analysed using a mixed model least-squares analysis for fitting constants (Harvey, 1990) including all main effects and interaction, to overcome the difficulty of disproportionate sub class number and non-orthogonality of data. In the initial model, some fixed effects and all 2-way interactions were found non-significant and these non-significant fixed effects and all interactions were ignored in the final model, which is as follows:

$$Y_{ijkmnp} = \mu+S_i+P_j+A_k+T_m+b(X_{ijkmn}-\mathbf{X})+e_{ijkmnp}$$

Where,

Y_{ijkmnp} is the record for the pth animal

μ is the overall mean,

S_i is the random effect of the ith sire

P_j is the fixed effect of the jth season of birth (j = 1, 2)

A_k is the effect of the kth physiological status of ewes (k = 1, 2, 3)

T_m is the effect of the mth birth status (m = 1, 2),

b is the linear regression coefficient for the age of animal

X_{ijkmn} is the record for the mth ewe

$\mathbf{X}$ is the mean for the trait

e_{ijkmnp} is the residual error elements with standard assumptions

The genetic and phenotypic parameters of various traits were estimated by the paternal half-sib method. The comparison of different sub-groups mean was made by Duncan's Multiple Range Test (DMRT) as described by Kramer (1957).

RESULTS AND DISCUSSION

Prevalence of nematode infection in lambs and ewes

The overall prevalence rate of gastrointestinal

Table 1. Least-squares means for $\log_e$ (FEC+100) [LFEC], PCV and body weight of ewes by seasons of birth, physiological status of ewes and birth status of animals at 2 sampling periods in Muzaffarnagari sheep

Parameters	No. of obs.	LFEC (GFEC)*	PCV (%)	Body weight (kg)
Overall mean	270	5.93±0.13 (276.15)	26.42±0.32	36.06±0.94
Season of birth				
March-April	138	6.17±0.149 ^{a} (378.18)	26.54±0.36	35.59±1.00
October-November	132	5.71±0.145 ^{b} (201.87)	26.39±0.35	36.54±0.97
Physiological status of ewe				
Dry	69	5.78±0.167^b (223.76)	27.58±0.4^b	35.37±1.12 ^{b}
Lactating	81	6.69±0.164 ^{a} (704.32)	24.28±0.39^a	35.31±1.03 ^{b}
Pregnant	120	5.35±0.149 ^{c} (110.61)	27.39±0.36^b	37.52±0.98 ^{a}
Birth status of animal				
Single	243	5.93±0.114 (276.15)	26.18±0.28	36.17±0.86
Twin	27	5.95±0.205 (283.75)	26.65±0.49	35.95±1.25

[a, b] Means with different superscripts vary significantly (p<0.05) from each other.
* Units eggs/g, the mean of the LFEC value is given first followed by its antilog in parentheses.

parasitism in lambs was 77.8% (i.e., 70/90). The major source of gastrointestinal parasitic infection in animals is mainly from contaminated pasture with gastrointestinal parasites in this climatic zone. Out of 77.8% lambs, the prevalence of coccidial oocyst was 100%, whereas only 15.7% lambs were positive for nematode infection particularly *H. contortus* indicating very low prevalence of fecal egg count in lambs up to one year of age. Hence the study revealed that the major proportion of the population was less sensitive to *H. contortus* infection up to 12 months of age in Muzaffarnagari lambs.

The prevalence of gastrointestinal infection in adult sheep was observed as 91.9% (i.e., 319/347). Out of 319 infected animals, a large proportion i.e., 67.7% of sheep was infected to *H. contortus* nematode infection and 89.97% was affected by coccidiosis infection. The investigation showed that the occurrence of the *H. contortus* infection was much higher in adults than lambs.

Factors affecting FEC, PCV and body weights of ewes

The least-squares means for log-transformed fecal egg count (LFEC), PCV and body weights of ewes in relation to season of birth, physiological status of animal and birth status (single vs. twin) have been presented in Table 1.

Factors affecting FEC : The overall least-squares mean for LFEC in ewes during the course of infection was 276.15±1.14. The random effect of sires contributed significant (p<0.01) variations on LFEC of ewes. The significant effect of sire on this trait indicated that superior rams could be used effectively for enhancing the resistance to nematode infection. Similar significant sire effect on fecal egg count was also reported by Romjali et al. (1997) and Gauly and Erhardt (2001) in different sheep breeds. The season of birth had significant (p<0.01) effect of LFEC of ewes (Table 1). The animals born in the month of October-November showed significantly lower (36.9%) fecal egg count in their adulthood as compared to adult animals born in the month of March-April. Similarly, Chauhan et al. (2003) reported that the kids born in the autumn (October-November) had significantly lower FEC as compared to kids born in spring (March-April) at 6 month of age in Jamunapari and Barbari goats in this region. However, Romjali et al. (1997) reported that the lambs born from February to March had lower fecal egg counts than those from May to June and August to September. The LFEC of ewes was also significantly (p<0.01) affected by physiological status of animals (i.e., dry, lactating and pregnant) in the present study. The lactating ewes had significantly higher fecal egg count as compared to dry and pregnant ewes. Significant (p<0.01) variation also exists between dry and pregnant ewes for this trait. Similar findings of higher fecal egg count in lactating animals were observed in different breeds of sheep (Barger, 1993; Romjali et al., 1997; Tembely et al., 1998; Baker, 1999). The high fecal egg count in lactating ewes may be due to poor nutrition, stress, lack of antigenic stimulation and hormonal suppression of immunity of which the latter is considered to be the most likely cause (Barger, 1993). The birth status of animals had no significant (p>0.05) effect on LFEC of ewes in this study. Non-significant effect of birth status on FEC was also reported by Gauly and Erhardt (2001) in Rhön sheep. However, Woolaston and Piper (1996) and Romjali et al. (1997) reported significant effect of birth status on FEC in sheep. The linear regression effect of the age of ewes on LFEC was negative (-0.001) and significant (p<0.05) in our study. The present findings were in agreement with those reported in other sheep breeds (Woolaston and Piper, 1996; Romjali et al., 1997; Gauly and Erhardt, 2001).

Factors affecting PCV : The overall least-squares mean for PCV of ewes was 26.42±0.32% during the course of infection. The sires of animals had significant effect (p<0.01) on PCV of animals. Significant sire variations for PCV were also observed by Gauly and Erhardt (2001) in Rhön sheep. Season of birth had no significant (p>0.05) role in variation of packed cell volume of the ewes (Table

Table 2. Estimates of heritability (diagonal), genetic correlations (below diagonal) and phenotypic correlations (above diagonal) of LFEC, PCV and BWT of Muzaffarnagari sheep

Parameters	LFEC	PCV	BWT
LFEC	0.24±0.17	-0.60	-0.06
PCV	-0.73±0.39	0.25±0.17	-0.01
BWT	-0.26±0.15	0.58±0.25	0.47±0.21

1). On the contrary, Romjali et al. (1997) found significant variations in PCV of animals born in different seasons. The physiological status of ewes had also significant ($p<0.01$) influence on PCV of ewes. The investigation showed that lactating ewes had lower PCV than dry and pregnant ewes whereas, the difference between PCV of dry and pregnant ewes was statistically non-significant ($p>0.05$). The lactating ewes had lower PCV as well as higher GFEC. Baker et al. (1999) studied the role of physiological status of ewes on fecal egg count and PCV of animals. They were of the opinion that there was a significant increase in FEC and decrease in PCV over the first two months of lactation in lactating ewes as compared to non-lactating ewes. The birth status and linear regression of age of ewes had non-significant effect on PCV of ewes. The non-significant effect of birth tatus on PCV was also reported by Gauly and Erhardt (2001) in Rhon sheep. However, significant variation in PCV due to birth status was observed by Romjali et al. (1997) in three genotypes of sheep. The significant effect of age of animals on PCV was reported by Romjali et al. (1997) and Gauly and Erhardt (2001) in sheep breeds, which is contrary to the present study.

Factors affecting adult body weights : The overall least-squares mean for body weights of ewes was 36.06±0.94 kg during the experimental period. The effect of sire and physiological status of animals were found significant ($p<0.01$) on body weights of animals. The regression of age of animal on body weight was also significant ($p<0.01$) in the present study. The various environmental factors (period, parity, sex, birth status) had significant effects on body weights at the various ages of growth in different breeds of sheep (Wilson et al., 1996; Yazdi et al., 1998).

Genetic and phenotypic parameter estimates

The heritabilities and genetic and phenotypic correlations estimates of LFEC, PCV and body weight are presented in Table 2. The heritabilities of all the traits under study were moderate to high in magnitude and ranged from 0.24 to 0.47. Genetically, the LFEC of ewes negatively and significantly ($p<0.05$) correlated with PCV of animals during the course of infection. Similarly, there was also significant ($p<0.05$) association between LFEC and PCV of animals at phenotypic level. The higher FEC was associated with lower PCV during infection in the current study suggests that the presence of more blood sucking parasites in gastrointestinal tract of animals causes the decrease in PCV of ewes. The study also revealed that the LFEC of animals had negative relationship with body weight (-0.26) of animals at genetic level. The moderate negative correlation between FEC and live weight of ewes indicate that resistance to gastro-intestinal parasites is probably an important determinant of growth rate in this environment. The phenotypic correlation between fecal egg count and live weight was very low (-0.06) and statistically non-significant ($p>0.05$) in our study. The live weight at any given age is a cumulative effect of all the experiences that the ewes had up to that age, whereas a fecal egg count simply describes the state of being at that point in time, for the current infection. The genetic correlation of PCV of animals with body weight was positive and high in magnitude and statistically significant ($p<0.05$) and it was negatively correlated with body weight (-0.01) at phenotypic level. Although the heritability estimates for transformed FEC of animals demonstrated in the present study are moderate, they are within range of those observed in other sheep breeds (Morris et al., 1997; Gauly and Erhardt, 2001; Gauly et al., 2002). In a study, Vanimisetti et al. (2003) estimated the heritability of FEC of animals infected with *H. contortus* in crossbred sheep (50% Dorset, 25% Rambouillet and 25% Finnsheep) and reported that heritability of mean FEC and log transformed FEC (LFEC) was 0.18 and 0.55 in ewes, respectively. The heritability of PCV ranged from 0.08 to 0.56 in different studies of many workers (Gauly and Erhardt, 2001; Gauly et al., 2002; Vanimisetti et al., 2004). The heritability estimate of PCV in the present study was well within the range of reported values. The low phenotypic correlation between egg count and live weight in current study (Table 2) was in agreement with most published results. Bisset et al. (1992) estimated the phenotypic correlation of $\log_e$ (FEC+1) with all production traits as negative and very low (-0.01 to -0.05) and their corresponding genetic correlations were also negative (-0.05 to -0.36). Bishop et al. (1996) also obtained negative phenotypic correlations between FEC and live weight in Scottish Blackface lambs, but the values were close to zero. The negative correlations of FEC and PCV, confirmed in a number of studies in different breeds, may be attributed to the fact that blood sucking parasites were dominant (Roberts and Swan, 1982). The selection based on FEC are probably more effective regarding the heritabilities and the fact, that in many studies FEC is highly correlated with the worm burden (McKenna, 1981; Eady, 1995) and the haematocrit is only a useful indicator if blood suckling parasites are dominant species.

CONCLUSION

The study revealed that the occurrence of *H. contortus* infection was more in adults than lambs in the flock of Muzaffarnagari sheep. The fecal egg counts and packed cell volumes were found to be good indicators of naturally infected nematode infection and egg counts were negatively correlated with packed cell volume of ewes infected with *H. contortus* parasite in this breed. The phenotypic correlations of fecal egg counts and packed cell volume with body weights were low and negative, indicating that mild influence of these parameters on body weight of ewes. The experimental evidence showed that the FEC can be used to assess the resistance/susceptibility status within this breed of sheep.

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