

Phenotypic Marker Based Evaluation of Resistance to *Haemonchus contortus* in Sri Lankan Indigenous Goats and Their Jannapari Crossbreds

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ABSTRACT: *The parasitic resistance in Sri Lankan indigenous (SLI) goats is getting gradually replaced owing to unsystematic crossbreeding. This study was conducted to identify phenotypic resistance to haemonchosis of between and within genotypes in SLI goats and their Jannapari crossbreds (JCB) and to identify level of phenotypic resistance to gastrointestinal strongyle parasitism exists in SLI and JCB at field. The study consists of an artificial challenge (40 goats) and a field trial (521 goats). In artificial challenge, two genotypes have responded to parasitism differently. Significant effects of measurement time (MT) and MT×genotype interaction were reported for log transformed faecal egg counts (FEC). Effect of MT was significant for least square mean packed cell volume (PCV). Peak FEC was reported at day-35 for both SLI and JCB; whereas, the lowest PCV values were observed for SLI and JCB at 28th and 42nd day respectively. When animals were grouped based on the highest recorded FEC, a majority of SLI (55%) was occurred under the category intermediate (I) while a majority of JCB (78%) was in the category high (H). Category low (L) was represented only by SLI (20%). Significant effects of group, MT and group × MT were observed on ln FEC in both genotypes. In both SLI-H and JCB-H, the highest FEC was reported at day-35. SLI-I and SLI-H were recovering from parasitism at the latter part of experiment whereas JCB-I and JCB-H were continuing with parasitism. In the field trial, both genotypes occurred in high frequency in group L. Hence, phenotypically SLI were more resistant to haemonchosis than JCB. There is a high within genotype variability for phenotypic parasitic resistance in both genotypes. Thus, this information is important in identifying genomic parasitic resistance in both genotypes.*

Keyword: *Artificial challenge, haemonchosis, indigenous goats, parasite resistance*

INTRODUCTION

Goat milk and meat play an important role in the nourishment of rural farm families where goat farming is predominant. However, goats are an underutilized genetic resource in Sri Lanka. Hence, it has a great potential for development (Ministry of Livestock and Rural

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Community Development, 2009). Since there is no wild relatives of domestic goats exist in Sri Lanka, all goats in the country are introduced animals (Chandrasiri, 2002; Abeyratne, 2007). Sri Lankan Indigenous (SLI) goats have been evolved within country for centuries adapting to parasites, pathogens and harsh environmental conditions exists at the field level (Ravindran *et al.*, 1983; Chandrasiri, 2004). In many parts of Sri Lanka, gene pool of SLI goats is getting replaced from imported exotics such as Jamnapari due to unsystematic crossbreeding. Therefore, currently, crossbred goats are the most common goat type in Sri Lanka. The unsystematic crossbreeding may have already reduced the frequencies of genes influencing on parasitic or disease resistance that SLI possessed. However, irrespective to the genotype, goats are mainly reared under extensive or semi-intensive management system exposing to pathogens and parasites indiscriminately (Silva *et al.*, 2009). Hence, identification of parasitic resistance exists within and between goat breeds and their crosses at the field level is important in improving the goat production in Sri Lanka. Except limited investigations on prevalence of parasitism (Faizal *et al.*, 1999; Rajapakse *et al.*, 2000; Faizal and Rajapakse, 2001), no systematic characterization of resistance to parasites has been conducted for SLI goats or their crossbreds. Therefore, this study was designed to identify between genotype and within genotype phenotypic resistance to haemonchosis in SLI goats and their crosses with Jamnapari (JCB) and to identify the level of phenotypic resistance to gastrointestinal (GI) strongyle parasitism exists in SLI and JCB goats under the field conditions of Sri Lanka.

MATERIALS AND METHODS

The experiment was designed as two-stage study including an artificial challenge trial and a field trial. The artificial challenge trial was designed to identify the phenotypic resistance to haemonchosis between and within SLI and JCB goat genotypes and the field trial was designed to identify the level of phenotypic resistance to GI strongyle parasitism exists in SLI and JCB goats under the field conditions of Sri Lanka.

Artificial challenge trial

The artificial challenge trial was conducted using two commonly exist goat genotypes in Sri Lanka namely, SLI and JCB goats.

Preparation of *H. contortus* L₃ larvae for artificial challenge trial

Female, adult *H. contortus* worms collected from goat abomasal mucosa obtained from goat slaughterhouse located at Kandy were washed and crushed using a mortar and pestle to collect parasite eggs. Parasitic eggs were cultured on sterilized cattle manure for three weeks at room temperature with a moisture level of 80 to 100% as explained by Hansen and Perry (1994). *H. contortus* L₃ larvae were recovered from the cultures after three weeks using Baerman technique (Hansen and Perry, 1994).

The recovered *H. contortus* L₃ larvae were used to artificially challenge two parasite-free, 3-month-old male donor goats at a dose of 5000 larvae. Donor goats were kept in an isolated environment and provided with *ad libitum* access to feed and water. After three weeks, all faecal matter excreted by the donor goats were collected using faecal collection bags continuously for one month. The faecal egg counts (FEC) was monitored by modified McMaster counting technique (Gordon Whitlock, 1939). When the FEC was higher than 1000 EPG the collected faecal matter was cultured on sterilized cattle manure on the same day of collection under the same conditions mentioned above. *H. contortus* L₃ larvae were recovered using Baerman technique from the cultures after three weeks, were used to artificially challenge the experimental goats.

Artificial challenge of experimental goats

The artificial challenge trial was conducted in the Karambewa area of Anuradhapura district from month of September to October in 2012. The site was located in the dry zone of Sri Lanka and the site selection was based on the fact that both SLI and JCB goats are commonly reared in the dry and dry-intermediate climatic zones in Sri Lanka.

Forty male goats including 20 SLI and 20 JCB goats were selected for the study based on the phenotypic characteristics of the animals and information provided by the Government Veterinary Office regarding the distribution of exotic breeds. The SLI goats were selected from seven farms in the Wahare and three farms in the Horowpothana veterinary ranges. The JCB goats were selected from seven different farms in Thirappane, Anuradhapura, and Puttalam veterinary ranges. All 40 goats were housed in a community goat house with a slatted floor. Feed and water were provided *ad libitum* during the experiment. Kids grazed during the day in a designated area and were supplemented with concentrates and tree fodder (*Gliricidia sepium*) during the night. All goats were dewormed using oral levamisole with a dose of 12 mg/kg body weight at the time of housing, and acclimatized for 1.5 months period before the experiment was commenced. Numbers of eggs in faecal samples were determined using modified McMaster counting technique described by Gordon and Whitlock (1939). Sampling was carried out at the time of housing and subsequently every two weeks to ensure that experimental goats were free from GI strongyle parasites. Each experimental goat was then artificially challenged with an oral dose of 5000 *H. contortus* L₃ larvae. The goats were approximately at 4 months of age at the time of challenged. Data collection was carried out for 42 days after artificial challenge, and on the day 42 the goats were dewormed with oral levamisole at the dose of 12 mg/kg body weight.

Data collection

At the time of challenge (day 0) and 21, 28, 35 and 42 days after challenge, blood and faecal samples were collected from each goat. Blood samples were collected from the external jugular vein to ethylenediaminetetraacetic acid (EDTA) tubes. The packed cell volume (PCV) was determined by the micro-haematocrit method (Jain, 1986). The faecal samples were collected directly from the rectum, The FEC was assessed using the modified McMaster technique where each egg observed was assumed to represent 50 EPG (Gordon and Whitlock, 1939).

Field trial

A total of 521, four to six months old goats, including 279 SLI goats (146 female and 133 male) and 242 JCB goats (111 female and 131 male) which are managed under similar management conditions were selected for the study. The SLI goats were selected from 34 farms located in Wahare and Kiran Veterinary ranges and JCB goats were selected from 24 farms located in Kalmunai, Addalachchenai, Samanthurai veterinary ranges of Eastern province. Blood samples and faecal samples were collected from each animal separately two times with seven days interval and analyzed as described above for PCV and FEC (Gordon and Whitlock, 1939; Jain, 1986).

Data analysis

One JCB goat was removed from the experiment on day 14, for reasons not related to parasitism. In addition, two JCB goats were judged to have a high risk of death based on clinical signs and low PCV hence, were removed during the experiment at day 21 and day 28. Weekly changes in FEC and PCV in SLI and JCB goats as a response to parasitic infection were analyzed with a repeated-measures analysis using the MIXED Procedure of

SAS 9.2 with a significance level of 95% unless otherwise indicated (SAS Inst., Inc., Cary, NC, USA). The model included fixed effects of genotype, measurement time (the repeated factor) and genotype by measurement time interaction and a random animal effect. Further, an unstructured covariance matrix was used for the analyses. Correlations between PCV and FEC were obtained by PROC GLM procedure of SAS 9.2.

In order to identify the within genotype variability of response to parasitic infection, the highest recorded individual FEC of each animal during the 42 days experimental period were plotted using Minitab 16 software. According to the peak FEC, animals were grouped (Fakae *et al.*, 2004) low “L” (<1000 EPG), intermediate “I” (1000 to 2500 EPG) and high “H” (>2500 EPG). The details of animal grouping and the respective frequencies of animals are presented under results and discussion section. Weekly changes in FEC and PCV within each genotype were analyzed with repeated measures of analysis using the MIXED procedure of SAS 9.2. The model includes fixed effects of group (L, I and H), measurement time (the repeated factor) and interaction effect of group×measurement time and a random animal effect.

Normality of FEC distributions were improved by transforming FEC of both trials as $\ln(\text{FEC} + 100)$. Resulting least-square means for $\ln(\text{FEC})$ were back-transformed for presentation as $e^{\text{LSM}} - 100$. Standard errors of least-square means were assumed to be approximately equal to coefficients of variation of back-transformed means, and standard error of back-transformed means were approximated by multiplying standard error of least-square means by back-transformed means. All FEC observations at day 0 were equal to zero and were not included in the analysis.

Similar to artificial challenge trial, goats of field trial were also classified into three groups L, I and H as described above. Animal frequencies of two genotypes in each group at the field level and the mean PCV for each group were obtained using Minitab 16 software.

RESULTS AND DISCUSSION

Phenotypic resistance to haemonchosis between SLI and JCB goat genotypes

The results of FEC showed that artificial challenge with *H. contortus*L₃ larvae has established the parasitic infestation in both SLI and JCB goats although two genotypes responded differently (Figure 1). Significant differences were observed in $\ln(\text{FEC})$ between the two genotypes ($p < 0.05$) measurement time ($p < 0.001$) and genotype×measurement time interaction ($p < 0.001$). The SLI goats were more susceptible to parasitic infection at the beginning of the experiment compared to JCB goats. In both groups, the peak values of FEC were observed at 35 days after the artificial challenge. However, SLI goats were able to recover from the parasitic infection without progressing to high FEC levels where JCB goats continued the progression. The FEC is widely considered as a simple and reliable phenotypic marker for identifying parasitic resistance in animals (Patterson *et al.*, 1996; Mandonnet *et al.*, 2001; Vegenas *et al.*, 2002). Further, many studies have reported low FEC as well as rapid recovery from initial elevations of FEC in parasitic resistant goats (Pralomkarn *et al.*, 1997; Makunet *et al.*, 2008; Chiejinaet *et al.*, 2015). Hence, FEC results of current study reflect that SLI goats are more resistant to haemonchosis than JCB goats during the artificial challenge.

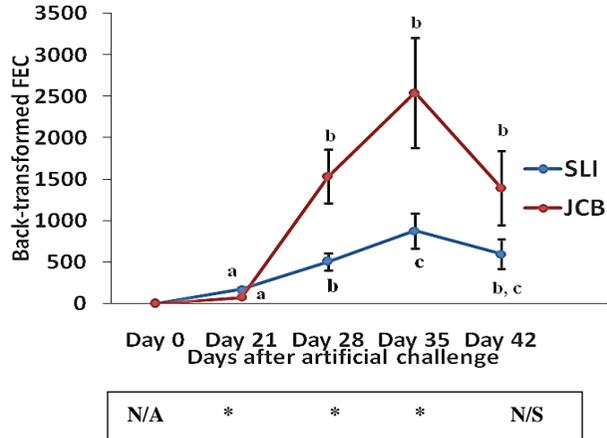


Figure 1. Least-square means for faecal egg counts (FEC) for Sri Lankan Indigenous (SLI) and Jamnapari crossbred (JCB) goats during artificial challenge trial

Note: Means for measurement times within each genotype with different letters were significantly different ($p < 0.05$). The significance of genotypic difference is indicated in the box below the graph (N/A = not tested; N/S = not significant; * = significant at $p < 0.05$).

Many studies have revealed that there is a strong correlation between FEC and haematological parameters where the reduction of PCV is a main character in haemonchosis (Blackburn *et al.*, 1991; Chiejina *et al.*, 2002; Fakaie *et al.*, 2004; Kaplan *et al.*, 2004; Khobra *et al.*, 2012; Chiejina *et al.*, 2015). Current study also confirmed that there is a significant negative correlation between FEC and PCV ($p < 0.05$). The analysis of haematological parameters revealed that there was no significant genotype and genotype \times measurement time interaction effect on PCV ($p > 0.05$). However, effect of measurement time on PCV was significant ($p < 0.05$). Although PCV has been significantly reduced with the onset of parasitism, the variation of PCV of SLI goats suggested that SLI animals were recovering from anaemia caused by haemonchosis at the end of study. In contrast, PCV values of JCB goats remained low suggesting that they were suffering from anaemia even at the end of experimental period (Figure 2). On the other hand, the experimental period of 42 days may not have been sufficiently long enough for JCB goats to recover from the parasitic infection. However, this reflects possibility of higher production losses due to long duration of parasitism in JCB goats compared to SLI goats.

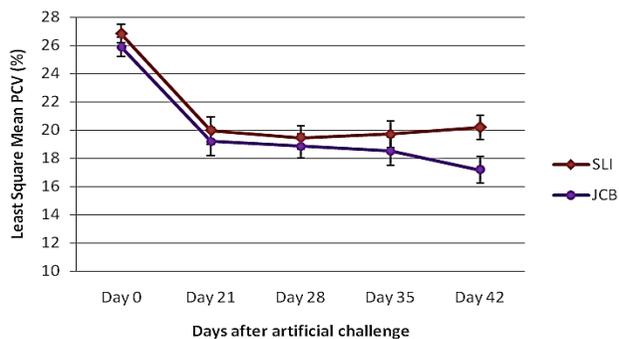


Figure 2. Change in PCV of SLI and JCB goats during the experimental period

Phenotypic resistance to haemonchosis within SLI and JCB goat genotypes

The Figure 3 depicts the dot plot distribution of the recorded highest FEC of individual animal during the experimental period. As shown, SLI goats were more resistant to haemonchosis compared to JCB goats since FEC of SLI goats have been clustered towards the low EPG counts. The dot plot distribution further justifies the identification of FEC classes in the current study, i.e. low (<1000), intermediate (1000 – 2500) and high (>2500), following Fakae *et al.* (2004)

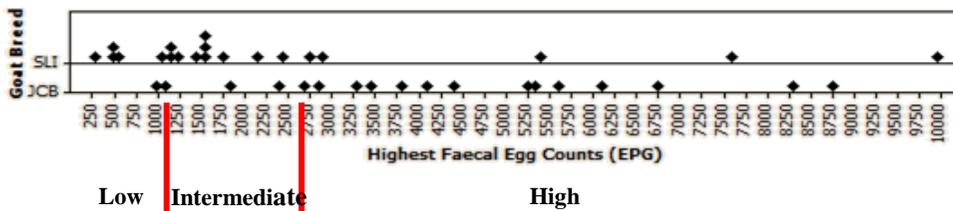


Figure 3. Dot plot distribution of highest recorded FEC of SLI and JCB goats during the experimental period

Majority of SLI goats (55%) belonged to the group I, whereas majority (78%) of JCB goats was occurred in the group H (Figure 4). Although 20% of SLI goats belonged to the group L, none of the JCB goats were reported in that category. The frequency distribution of two goat genotypes among the three FEC classes exhibited the existence of considerably high within genotype variability where SLI showed greater variation with higher resistance similar to the cases reported for West African Dwarf goats (Fakae *et al.*, 1999; Chiejina *et al.*, 2005; Behnke *et al.*, 2006).

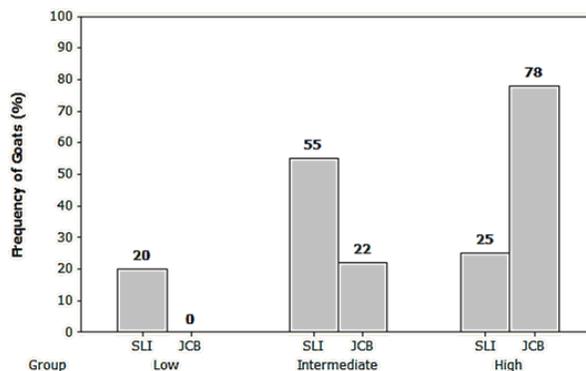


Figure 4. Frequency of SLI and JCB goats belonging to different groups in artificial challenge trial

Both SLI and JCB goats have developed parasitic infection at the day 21 and FEC were similar in all groups within the genotype ($p > 0.05$). There was a significant group, measurement time and group×measurement time interaction effect on ln (FEC) in both genotypes. SLI goats in group L have maintained the FEC at a very low level throughout the experimental period (Table 1). Groups I and H of both genotypes showed increasing trend of infection until day 35 post challenge, where peak FEC was observed. Although SLI goats in FEC group H showed a significantly high FEC at the day 35 compared to group I, both

groups seemed to be recovering from parasitism showing significantly lower FEC at the day 42 than that at the day 35 post challenge ($p < 0.05$). After the peak FEC at the day 35, JCB goats in both I and H groups also showed a reduction in FECal though not significant ($p > 0.05$).

There was a significant effect of measurement time on PCV in both SLI and JCB goats ($p < 0.05$). The analysis of within genotype variability of PCV in SLI goats have reported the highest level of anaemia, i.e. $PCV=16.20\pm 1.53\%$ in the group H at the day 28 (Table 1). However, they seemed to be recovering from anaemia at the day 42 post challenge having statistically similar PCV to the day 21 of experiment. The highest level of anaemia, i.e. $PCV=17.12\pm 1.07\%$ in JCB goats was reported in the group H at the day 42. Although statically not significant, declining trend in PCV of JCB goats in group H is continuing even at the day 42 post challenge confirming the results of FEC. However, in both genotypes group H has reported the highest level of infection reflecting a potential of considerable production losses in the group H. High within genotype variability for haemonchotolerance have been reported from West African Dwarf goats by Fakae *et al.* (2004) and Chiejina *et al.* (2005). Similarly, the observed differences in PCV and FEC results among three groups of goats in the current study are possibly due to differential expression of haemonchotolerance within SLI and JCB goats.

Table 1. Least square means (LSM± Standard error (SE)) of faecal egg count and packed cell volume in different groups of SLI and JCB goats during the experimental period

Parameter	Genotype	Group	Day 0 (LSM±SE)	Day 21 (LSM±SE)	Day 28 (LSM±SE)	Day 35 (LSM±SE)	Day 42 (LSM±SE)
FEC (EPG)	SLI	L	0	105.9±39.4 ^{a,*}	169.1±37.0 ^{a,*}	91.6±32.0 ^{a,*}	411.1±277.9 ^{a,*}
		I	0	182.0±40.8 ^{a,*}	489.9±64.6 ^{b,**}	825.5±174.1 ^{c,**}	662.0±269.9 ^{a,b,c,*}
		H	0	218.9±72.8 ^{a,*}	1170.1±229.1 ^{b,+}	4080.1±1276.6 ^{c,+}	643.3±389.0 ^{a,b,*}
	JCB	I	0	39.1±9.4 ^{a,*}	502.3±236.2 ^{b,*}	1036.7±346.0 ^{b,*}	426.0±275.2 ^{b,*}
		H	0	74.7±9.6 ^{a,*}	2018.1±507.5 ^{b,+}	2997.3±550.3 ^{b,+}	1897.4±675.6 ^{b,*}
		L	25.7±1.6 ^{a,*}	19.8±2.1 ^{b,*}	21.1±1.7 ^{b,*}	19.1±2.1 ^{b,*}	18.1±1.9 ^{b,*}
PCV (%)	SLI	I	27.3±0.9 ^{a,*}	20.9±1.3 ^{b,*}	20.3±1.0 ^{b,*}	20.8±1.3 ^{b,*}	21.1±1.1 ^{b,*}
		H	26.5±1.4 ^{a,*}	18.8±1.9 ^{b,c,*}	16.2±1.5 ^{b,+}	17.7±1.9 ^{b,*}	19.8±1.7 ^{c,*}
		I	24.5±1.0 ^{a,*}	20.8±2.0 ^{b,*}	20.2±1.7 ^{b,*}	21.4±1.8 ^{a,b,*}	18.8±1.9 ^{b,*}
	JCB	H	27.0±0.5 ^{a,+}	19.5±1.1 ^{b,*}	19.0±0.9 ^{b,*}	18.3±0.9 ^{b,*}	17.1±1.0 ^{b,*}

Note: Least square means with different superscript letters within a row and the LSM with different symbols between groups of each genotype for a given parameter differ significantly ($p < 0.05$)

Phenotypic resistance to gastrointestinal strongyle parasitism in SLI and JCB goats under the field conditions of Sri Lanka

The results of the field trial revealed that majority of SLI (68.7%) and JCB (62.4%) goats belong to the group L (Figure 5). However, frequency of goats in group H is high in JCB goats (16.9%) compared to SLI goats (6.1%). Since the stage of infection in the goats at the field level is unknown in the current study, the actual frequencies of each category can be different from the values of current study. PCV of L, I and H groups of SLI goats were 24.37 ± 0.28 , 22.71 ± 0.40 and 21.59 ± 1.02 , respectively and those of JCB goats were 23.78 ± 0.27 , 22.64 ± 0.53 and 22.34 ± 0.50 , respectively. However, current study reflects that although uncontrolled breeding occurs at the field level and important characters such as parasitic resistance is believed to be replaced from exotics, still there is a considerable proportion of phenotypic parasitic resistance exists among goats at the field level of Sri Lanka.

Although FEC is considered as a phenotypic marker, the major limitation in using FEC as the key criteria for selecting animals for haemonchotolerance is that high levels of FEC can be observed only after establishment of parasitic infection. By the time when such observation is made, the damage might be irreversible (Fakae *et al.*, 2004). Further, Fakae *et al.* (2004) suggested that host acquired immunity may have significant influence on the resistant status of goats. As revealed by many studies, genetic characterization of parasitic resistance in goats will be important in selection of animals for breeding (Pralomkarn *et al.*, 1997; Baker *et al.*, 1998). Hence, genetic characterization of goats belong to different phenotypic classes of current study will be important in selective breeding of Sri Lankan goats for resistant to GI nematodes. The current study will provide basic information for identifying the resistant animals within the two goat genotypes. Further, current study gives a positive indication that although uncontrolled breeding has replaced a part of indigenous goat genetic pool, still it is not too late to implement genetic conservation attempt for parasitic resistant character by conducting proper selection and breeding program.

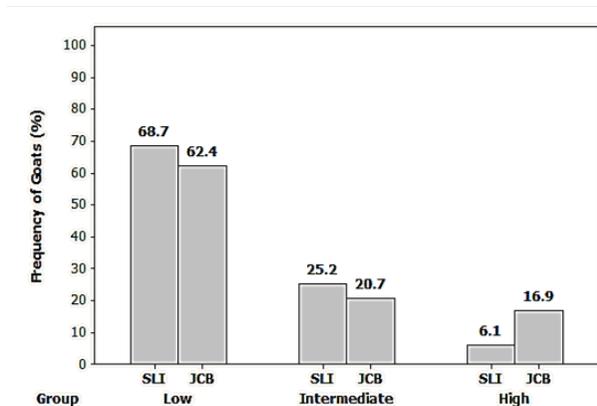


Figure 5. Frequency of SLI and JCB goats belonging to different FEC classes in field trial

CONCLUSIONS

SLI goats are more resistant to haemonchosis than JCB goats. However, phenotypically there is a within genotype variability for parasitic resistance in both SLI and JCB goats. There is a high level of phenotypic resistance for GI strongyle infection still exists among goats at the field level of Sri Lanka.

FUTURE DIRECTIONS

The phenotypic information of the current study provides important directive if coupled with the genetic information in order to select parasitic resistant goats for future breeding programmes.

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