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GENETIC VARIATION IN RESISTANCE TO CAPRINE FOOT ROT BY *Dichelobacter nodosus* IN GOATS OF KERALA, INDIA

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Abstract: Foot rot is a highly contagious and economically important disease of sheep and goats, caused predominantly by *Dichelobacter nodosus*. The current investigation was intended to analyse the genetic variation for resistance to caprine foot rot among two purebred native breeds of goats(Malabari and Attappady Black) and crossbred (Malabari crosses with Sannen, Alpine and Boer) goats in Kerala state, India. The cases were identified by observing characteristic symptoms of foot rot in goats, detecting Gram negative large rods from the hoof lesions and by PCR to detect the 783bp amplicon from the 16sRNA gene of *D. nodosus*. Two hundred and four animals were subjected to the study and statistical analysis of the data generated could substantiate that, there is variation in caprine foot rot resistance among genetic groups studied ($p \leq 0.01$) with significantly lower incidence rates in Malabari (14.29%) and Attappady Black (2.29%) compared to the crossbreds (43.75%).

Key words: Attappady Black, caprine foot rot, *Dichelobacter nodosus*, genetic variation, Malabari, resistance

Introduction

Goats are considered as an economically important livestock species in India on account of their short generation interval, higher prolificacy and excellent market potential. In southern states of India like Kerala, goats contribute considerably to the rural economy. Infectious diseases in goats could be regarded as the major cause of production loss, among which those associated with foot are of special importance as the goats are considered to be voracious grazing animals. Foot rot in domestic sheep and goats is a highly contagious disease that results from a mixed bacterial infection of the hoof, in which the obligate parasite *D.*

nodosus is essential for the initiation and establishment of the infection *(Hindmarsh and Fraser, 1985)*. Foot rot is a major concern in northern part of India *(Wani et al., 2004)* with no reports from the southern parts of India, though it is a well known disease of goats world over. The variation in the susceptibility to the disease among breeds may be attributed to the genetic differences in the goat breeds, which are predominantly seen in the native regions. Therefore a work plan has been made to analyze the genetic variation for resistance to caprine foot rot caused by *D. nodosus* among two purebred native breeds (Malabari and Attappady Black) and crossbreds (Malabari crosses with Sannen, Alpine and Boer) from a herd infection of foot rot in University Goat and Sheep farm, Mannuthy, Kerala, India.

Materials and Methods

Animals. Two hundred and four adult goats belonging to three genetic groups, Malabari (63), Attappady Black (45) and Malabari crosses with Sannen, Alpine and Boer (96) maintained at University Goat and Sheep farm, Mannuthy, Kerala were subjected for the study for a period from September to November 2010. The animals were managed through semi intensive system and were usually released for grazing for a minimum of 4 hours daily. They were also provided with a concentrate feed at the rate of 400g/head/day, with mineral supplements at the rate of 600g/100Kg feed/day and fodder grass adlibitum. The three genetic groups under the study were housed in independent pens on raised wooden platforms which were adequately ventilated and optimally illuminated. During the period of illness/ period of study the animals were thoroughly observed for the clinical signs associated with foot rot viz., limping, holding of limbs above the ground, reluctance to walk, presence of dark grey exudate and foul smell from the interdigital spaces *(Stewart et al., 1986)*.

Collection of samples and detection of the pathogen. Exudates/ tissues from foot rot lesions were collected from the goats showing the above clinical signs at random. Gram's staining of the samples revealed large Gram negative rods. The samples were processed to obtain the DNA of the pathogen by crude method i.e., the samples were suspended in 50µl sterile distilled water, boiled for 10 min, snap chilled on ice for 5min and centrifuged at 15000 rpm for 10 min and the supernatant was collected. The DNA obtained was employed in PCR, as it was reported that the PCR based methods using 16 sRNA gene specific primers have been used for the rapid diagnosis of D*. nodosus* from clinical samples *(La Fontaine et al., 1993; Wani et al., 2004; Moore et al., 2005; Wani et al., 2007)*.

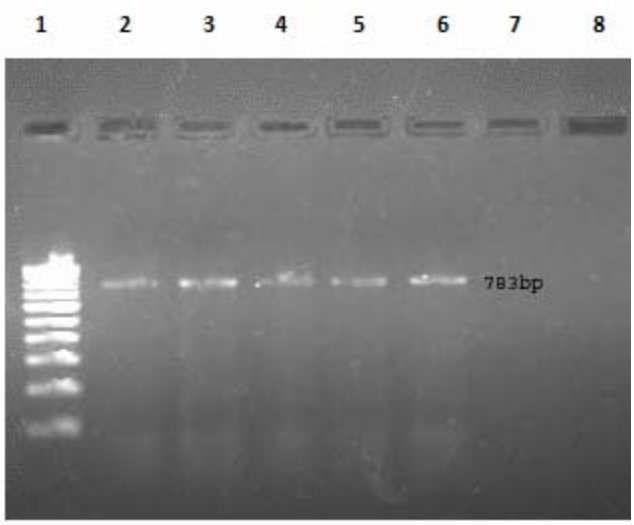
PCR amplifications were carried out in 25µl in 0.2ml thin walled PCR tubes. The PCR mixture contained a final concentration of 5µl of template, 2.5µl of 1X taq buffer (10mM Tris-HCl (pH 9.0), 50mM KCl, 15mM $MgCl_2$), 25pM of forward primer -5' CGGGGTTATGTAG CTTGC 3' and 25pM of reverse primer

-5'TCGGTACCGAGTATTTCTACCCAACACCT 3' (*La Fontaine et al. 1993*), 200µM of each deoxyribonucleotide triphosphate and 1U Taq DNA polymerase. The amplification was carried out in a Thermal Cycler consisted of 94^0C 10 minutes followed by 30 cycles of 94^0C for 1 minute, 58^0C for 30 seconds and 72^0C for 30 seconds and final extension at 72^0C for 5 minutes. PCR products were electrophoresed in 1.5% of Agarose gels, stained with ethidium bromide and visualized under ultraviolet (UV) illumination and photographed with gel documentation system.

Statistical analysis. The significance of genetic group on the incidence of caprine foot rot by *D. nodosus* was tested using Chi-square test. Further the significance difference in proportions of infected animals in each genetic group was tested using Z-test *(Snedecor and Cochran, 1994)*.

Results and Discussion

Among the two hundred and four goats subjected to the study, nine Malabari goats, four Attappady Black goats and 42 crossbred goats were showed the typical symptoms of foot rot. The randomly collected samples yielded PCR amplified products of an expected size of 783 bp, suggestive of 16sRNA gene of *D. nodosus* (Figure 1).



Lane 1 : 100bp DNA ladder
Lane 2-6: 783bp 16sRNA gene of *D. nodosus*
Lane 7,8: Negative controls

Figure 1. PCR amplification of 16sRNA gene of *D. nodosus*

The proportion affected in the three different genetic groups and the comparisons among them are presented in Table 1. The statistical analysis revealed a significant effect for genetic group in the incidence of caprine foot rot by *D. nodosus*, i.e., the incidence in Malabari and Attappady Black goats were significantly lower when compared to crossbreds. Malabari and Attappady Black are the indigenous breeds of Kerala which are well adapted to the local climatic conditions.

Table 1. Proportion of goats affected with foot rot in different genetic groups

Genetic group	Proportion affected	χ^2 value
Malabari	14.29[a]	24.34**
Attappady black	8.89[a]	
Crossbred	43.75[b]	

(** p≤0.01) (Superscripts with different alphabets differ significantly)

In Kerala, goat production is one of the important farm activities for the rural livelihood security. Foot rot is a costly disease in ruminant livestock population particularly during the wet season. Costs of labour, drugs, equipment and treatment, decreased flock productivity, losses from sales of breeding stock etc. make caprine foot rot, a disease of economic importance for producers *(Adama and Kudu, 2008)*. Though goats are very much susceptible to this disease, the susceptibility spectrum varies with different localities/ breeds *(Emery et al., 1984; Shimshony, 1989)*. Therefore, the present study envisages to study whether there is any difference in the susceptibility pattern to this particular disease among the various breeds commonly available in Kerala state.

Research on breeding for disease resistance in goats is very limited in India and disease resistance has not yet been included in breeding programs. There is well-documented evidence for within and between breed genetic variation in resistance to infectious diseases, namely gastrointestinal nematode infections, diseases due to mycotoxins, bacterial diseases including foot root and mastitis, ectoparasites such as flies and lice, and scrapie, the small ruminant transmissible spongiform encephalopathy *(Bishop and Morris, 2007)*.

Conclusion

Though foot rot can be controlled in an effective way through good management practices like the foot bathing, quarantine of the affected animals and vaccinations, adopting them in disease endemic area can make the economics of goat rearing to the non profitable side. Therefore, there is a need for a sustainable and long term solution like building up a flock with genetic resistance to foot rot in

areas where the disease is endemic. The lower incidence rate of caprine foot rot in the purebreds of Kerala as indicated in this study should be extrapolated to its maximum to develop strategies through traditional selection and molecular genetic testing methods to breed for resistance to foot rot in goats of Kerala. The genetic management of disease includes choosing an appropriate breed for the production environment or cross breeding to introduce genes of disease resistance, into a genetic group, which are otherwise well adapted for the required purpose, which is more applicable here. Since the crossbreds excel in their milk production and growth rate when compared to the other two groups, further marker studies are required to introduce foot rot resistant genes of native breeds into the crossbred blood.

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Genetska varijacija u otpornosti na koziju zaraznu šepavost prouzrokovanu sa *Dichelobacter nodosus* kod koza u državi Kerala, Indija

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Rezime

Zarazna šepavost je izuzetno zarazna i ekonomski važna bolest koja pogađa ovde i koze, a izaziva je bakterija *Dichelobacter nodosus*. Istraživanje je imalo za cilj analizu genetske varijacije u pogledu otpornosti na koziju zaraznu šepavost kod dve autohtone rase koze (Malabari i Attappady Black) kao i meleza (melezi Malabari i sanske, alpino i boer rase), u državi Kerala u Indiji. Slučajevi su identifikovani opservacijom karakterističnih simptoma ove bolesti kod koza, otkirvanjem gram negativnih velikih štapićastih bakterija iz lezija na papcima i PCR za otkrivanje 783bp amplikona iz 16sRNA gena *D. nodosus*. U ispitivanje su bile uključene 204 životinje, a statistička analiza dobijenih podataka je potvrdila da postoji varijacija u otpornosti na koziju zaraznu šepavost među ispitivanim genetskim grupama ($p \leq 0.01$), sa signifikantno manjim brojem slučajeva ove bolesti

kod malabri (14.29%) i Attappady Black (2.29%) u poređenju sa melezima (43.75%).

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