Prevalence, identification and molecular characterization of gastrointestinal nematode parasites of goat

SUMMARY OF THESIS

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SAVITA

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PROF. KAMAL JAISWAL

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करूणा
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DEPARTMENT OF APPLIED ANIMAL SCIENCES
SCHOOL FOR BIOSCIENCES AND BIOTECHNOLOGY
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226025,
UTTAR PRADESH, INDIA

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SUMMARY

Goat farming has an important role in rural communities around the world as well as being socially and economically highly significant at national and international levels. India has a first rank in goat farming, having largest goat population (117 million) and meat production (0.5%) in the world. In the national economy of India the goat farming plays a most important role. India has also contributed in the improvement of animal production and international livestock gene pool (Oldham *et al*., 2014). About 20.5 million populations depend upon livestock for livelihood and also contributed 14% and 16% to the income of small farms as against all rural households (Economic Survey Annual Report, 2018 to 2019). Two-third part of rural community depends on the livestock and also provides employment (8.8% population) in India. Livestock sector contributes 25.6% of total agriculture GDP and 4.11% GDP (Economic survey, Annual Report 2018 to 2019).

Uttar Pradesh, stands 2nd in the country with 11.53% contributed towards meat production. The Goat contributes about 15% of total meat production of Uttar Pradesh. Subclinical and chronic conditions resulting from reduced feed intake and decreased feed utilization efficiency are believed to be the major economic losses caused by nematode infections. There are lot of risk factors that cause losses in the goat farming, such as the some disease cause economical losses because of reduced growth productivity, immune suppression and milk production. Disease producing organisms are viruses, bacteria, protozoa, helminths etc.

Helminthiasis is biggest problem in the world and also leads to retardation in small ruminants. Helminths or worms cause a wide range of health problems to both humanbeings and animals. Helminths are classified into nematode, trematode and cestode. Helminth parasitic infections remain a major problem to small ruminant production in tropics and subtropical regions. They majorly belong to endoparasites found in gastrointestinal tract of goat. Helminthic parasite infestation is the greatest significant preventive aspect of production on the goat industry due to the morbidity, cost of treatment and mortality.

Gastrointestinal nematode parasitic infection is a major menace to the goat productivity and endangers for animal welfare worldwide. Among the gastro-intestinal

nematode parasitic diseases, such as *Haemonchus* sp.*Oesophagostomum*, sp. and *Trichuris*sp. are most common infectious diseases associated with anaemia, gastroenteritis resulting loss of body weight, stunted growth, bootle jaw and diarrhoea etc. that greatly weigh down the normal growth and production of goats.

Therefore this study determined the prevalence of gastrointestinal nematode parasites of the goat, and investigated the main risk factor associated with gastrointestinal nematode infection. Present study determined the prevalence with respect to month, age and gender, identification and molecular characterization of gastrointestinal nematode parasites in goat.

The first chapter of the thesis includes brief description on the importance of livestock in the rural economy, parasitism its origin, morphology, life cycle, and infestation of helminth infection in domestic animals. The method of diagnosis by microscopy and molecular chectarization techniques of the nematode species *Haemonchus* sp.*Oesophagostomum* sp. and *Trichuris*sp. also has been reported in this chapter.

The second chapter includes the literature review highlighting parasite identification, prevalence, morphological description, molecular characterization and impact of parasitic infestation on their hosts.

The third chapter study revealed the epidemiological data of gastrointestinal helminth parasitic infection in goats across various parameters during the study period of May 2016 to April 2019. The data was collected from different regions of Lucknow (Uttar Pradesh, India).

The overall prevalence of gastrointestinal parasites of goats were found to be 63.33% out of total 540, during the 3 years (May, 2016 to April 2019), collected from the gastrointestinal tracts of goat. During the study period, it was found that 369 gastrointestinal tracts were infected and 117 tracts were non-infected. The overall prevalence of gastrointestinal parasites was found that nonsignificant data (X^2 =.157, and $p>0.05$). The prevalence of gastrointestinal parasites infection was higher in goats than other ruminant animals it may be due to lack of proper immunity in the host against parasitic infection. Hot and humid conditions are providing more favourable conditions for the growth and development of infective worm.

Month wise prevalence oh helminth parasites in gastrointestinal tracts of goat was studied during 2016 to 2019. Higher prevalence of parasitic infection was found during July (94.11%) August (98.18%) and September (94.33 %) and lowest parasitic infection was found in months of January (20.00%), February (32.55%), March (58.69%) December (29.03%), which might be due to rainfall, hot and humid climatic conditions of study area that favours the development of larva of gastrointestinal parasites. Malnutrition and weak immunity of the goat, may also be responsible for the higher rate of prevalence and data was showed higher significance (X^2 = 146.94 and p <0.05).

Climatic factors depend on meteorological data, which shows highest and lowest temperature and humidity, respectively. The results also showed the (av. max and av. mini.) in the month of May (41°C. and 30°C) June (42°C and 33°C) and July (43°C and 35°C) and lowest temperature seen in the months of December (25°C and 16°C) January (23°C and 16°C) and February (27°C and 15°C). Humidity was evident (av. max and av. mini.) in the months of July (63% and 51%), August (71% and 50%) and September (63% and 52%) and lowest in the month of March (27% and 22%), April (22% and 18%) and May (25% and 19%). Changes in climatic factors might have direct effect on environment, pasture management indicating those region with increased or a decreased prevalence of the parasitic infection, abundance, and population dynamics (depending on the species).

The comparison study revealed prevalence of helminth parasites (nematode, trematode, cestode and mixed) month wise (January 2016 to December 2019). In the present study it was found that nematode parasites were most prevalent in the months of July, August and September (53.13%, 58.18% and 54.71%) and lowest in January, February and December (15.00%, 20.93% and 6.45%). Trematode infection was highly evident in the months of May, June and August (8.69%, 12.24% and 09.09%) and lower in Febuary, March, April, November and December (2.32%, 6.52%, 2.22%. 2.77% and 3.22%). Cestode was recorded higher in August and September (11.32% and 20.00%) and lowers in months of January, February and December (2.5% and 2.77%). It was observed that Nematode parasites (37.77%) are most prevalent parasites compared to trematode (05.73%) and Cestode (7.03%) in the study area. Nematodes were found to be the most prevalent than the other parasites and this might be due to geoclimatic conditions including climatic factors, optimal conditions (temperature and moisture), and nutrition

affecting the immune system and poor pasture and rearing pattern of the goats in the study areas. Statistical analysis data was recorded (X^2 = 64.62, and p<0.05 Significant for nematode), (X^2 = 16.08, and p>0.05 Non-significant for trematode), (X^2 = 13.08, and p>0.05 Non-significant for cestode) and (X^2 = 17.76, and p>0.05 Non-significant for mixed infection).

Season-wise prevalence of helminths parasitic infection was highly significant. However higher infection rate was detected during summer and monsoon season (71.50%, 87.25%) but lower in winter (33.33%). Season wise study was found with non significant data (X^2 = 127.96, and p>0.05 Significant). Nematode infections were also observed higher during the months of summer, monsoon and winters (44.08%, 50.98% and 16.00%), as compared to trematode (7.52%, 11.72%, and 4.00%), cestode (6.00%, 9.80% and 5.33%) and mixed infection (14.50%, 18.00% and 10.66%), respectively. However, variations in season-wise prevalence of parasitic infestation in goats was found significant (X^2=119.46, p<0.05 for summer, X^2=129.79, p<0.05 for monsoon and X^2 =20.81, p< 0.05 for winter). The increase in their prevalence during summer could be due to increase in humidity and availability of favourable temperature. Heavy rainfall and humidity are inclined to heavy parasitic infection.

The species-wise study revealed that out of 540 goats, 369 (68.33%) were infected by gastrointestinal parasites during 2016 to 2019. The identified gastrointestinal parasites were *Haemonchus* sp., (376/440), *Oesophagostomum* sp., (296/540) *Trichuris* sp., (246/540), *Paramphistomum* sp., (159/540) and *Moniezia* sp. (131/540). *Haemonchus* sp., (69.62 %) *Oesophagostomum* sp., (54.81%) *Trichuris* sp., (45.55 %) are nematode parasites, *Paramphistomum* sp. (29.44 %) is a trematode parasite and *Moniezia* sp. (24.25 %) is a cestode parasite. Nematodes were prevalent parasites in this study area showed that *Haemonchus* sp. is the most dominant parasites as compared to other parasites. Highest mean Intensity is prevalent in the *Parasphistomum* sp. (19.01) and *Haemonchus* sp. (13.39) when compared to other parasites (*Oesophagostomum* sp. (09.55) *Trichuries* sp. (09.12) and *Moninezia* sp. (02.00). Comparative study also showed highest abundance of parasitic infection in *Haemonchus* sp. *(*09.32*)* *Oesophagostomu* sp. (05.23) *Paramphistomum* sp. (5.29) than in *Trichuries* sp. (04.15) and *Moninezia* sp. (0.10). People therefore should be made aware trained to minimize the risk. The parasite

Haemonchus sp. was found most prevalent. Statistical analysis data was found significantly higher (X^2 =300.34 and p>0.05).

Age-wise parasitic prevalence revealed that the overall gastrointestinal parasitic infection was observed slightly in (> 6 month) Group-1 (74.48%), followed by 6 months to 12 month (Group-2) (57.14%) and <12 month (Group-3) (71.34%). In the present study, it was observed that Group-1 and Group-2 are very prone to gastrointestinal parasites infestation as compare to Group-3. Age wise study was found with significant data (X^2 = 10.90, and p<0.05 Significant).

In the present study, a total of 540 intestines inclusive of 293 female and 247 male goats were examined, out of which 208 female goat and 161 male cattle were found to be infested with gastrointestinal parasitic infection. The prevalence rate of parasites was found to be higher in female goats (70.98%) than the males (65.18%). However, variations in prevalence of parasitic infestation in goat was found with significant data (χ2-2.09 and p>0.05)

During the investigation, it was observed that gastrointestinal parasitic infection is highly dependent on good (18.95%), moderate (28.14%) and poor body condition (40.18%) of the goat and also significant prevalence (X^2 = 18.62 and p<0.05) of infection is dependent on the body condition of goat. Highest parasitic prevalence was found in the goat with poor body followed by moderate and good body conditions of the host**.**

The faecal examination showed high frequency of nematode eggs counts in host. Faecal (EPG) examination indicated that the highest parasitic burden in poor condition, 244/540 (45.18%) when compared to the moderate 118/540 (21.85%) and good condition 7/540 (1.29%) of gastrointestinal tract. Statistical data recorded revealed higher significant variations in this study (χ2=5.99 and p>0.05).

The fourth chapter study revealed about the causes of parasitic infection by *Haemonchus* spp.*, Oesphagostomum* spp.*,* and *Trichuries* spp. etc. and mixed infections were the most prevalent. The definitive classification is based on the external and internal morphology of egg, larval, and adult stages (Gilbert, 1996). Morphological identification of parasites were reported by Soulsby (1965). Observations of the parasites were total body length, spicules, cervical papillae, vulval flap and cervical lamina after preparing

mounted slides (Patchamuthu, 1993). Identification of individual worms was done with the help of Microscope and Scanning Electron Microscope (Rahman and Hamid 2007). Ultrastruture of nematode parasites and their eggs were determined using Compound microscope and SEM, its classification was examined based on species specific characters, shape and size of parasites and their eggs.

The five chapter revealed about the molecular characterization of nematode parasites species (*Haemonchus contortus, Trichuri ovis* and *Oesophagostomum columbianum*) by molecular technique (PCR, DNA sequencing and bioinformatics). The amplified PCR product range of approximately 325bp, 205bp and 117bp (Figure no-5.3 and 5.4). The generated sequence was blasted (BLAST) in nucleotide and compared to different accession number which showed homology (97% to 100%), which was future identified for of all nematodes using bioinformatics methods (BLAST, Clastal W, Oligo analyzer and Mega 7.0 software) and generating sequence (OES3.1). The phylogenetic tree analysis showed relationship between various species of parasites.

Significance and Future aspects

A significant aspect of studying the prevalence, distribution, and seasonal patterns of transmission of diseases in different climatic zones and improving treatment and control is taken up in the current research. Molecular characterization is a specific diagnosis method to identify nematode infections for refined investigations of parasite epidemiology. In future, these methods, and high-performance field-based assays and sequencing technologies will provide better prediction for disease surveillance and control programs against nematodiasis. Immunology and molecular based technique can be widely used for understanding the helminth parasites.