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PREVALENCE AND DNA BARCODING OF GASTRO INTESTINAL HELMINTHS IN *Gallus domesticus* FROM HIGH LAND REGIONS OF KOLLAM DISTRICT, KERALA

JEENA PRAKASH[1*] AND S. SYNUDEEN SAHIB[1]
[1]Department of Zoology, University of Kerala, Kariavattom, Thiruvananthapuram, India.

AUTHORS' CONTRIBUTIONS
This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Parasitic helminthes in birds can lead to decreased production of *Gallus domestica*. This study was carried out to assess the prevalence and analyse the DNA bar coding of parasite helminths in gastrointestinal tract of *Gallus domesticus* from highland regions of Kollam District. The study lasted for a period of eight months (from October 2017 to May 2018). For this study, 160 indigenous chicken's gastrointestinal tract of the birds were collected from freshly slaughtered birds from local poultry shops, markets, and household in the rural areas of the highland regions of Kollam District. The highest prevalence of parasite helminths of 65% was noted in the month of April and lowest prevalence of 40% in October. Based on the DNA bar coding of the samples, the parasitic phylogeny study was analysed. The nucleotide sequence data was analysed by the pair wise alignment tool blast N, the hits were compared based on the query coverage and percentage of identity. The major helminths parasites found in *Gallus domesticus* were *Ascaridia spp, Syngamous trachea spp, Raillietina spp*.

Keywords: Prevalence; parasite helminthes; *Gallus domesticus*; DNA barcoding.

1. INTRODUCTION

Poultry development has become one of the most important platforms to the farmers. The use of modern technique highlights the need to improve in the development of poultry industry. Poultry development has been a household activity in India. However, scientific poultry production in India gained momentum during the last four decades due to concerted efforts of the Government of India through policies, focused research and the initiatives taken by the private sector. The poultry sector has emerged from entirely unorganized farming practice to commercial production system with state-of-the-art technological interventions. Poultry sector, besides providing direct or indirect employment to people, is also a potent tool for subsidiary income generation for many landless and marginal farmers. It also provides nutritional security especially to the rural poor. As per 19th census in All India Livestock Census for 2012 indicated that poultry product constituted 95%, ducks 3% and others made up 2%. Apart from meeting up with local demands, the poultry products are also being exported from Kerala. As per the census in 19th Live Stock of poultry products of Kerala (2012) Rs. 242.82 lakh earning was recorded from this business and it shows the 3.3% of total poultry production in India. The occurrence of both ecto and endoparsites of

**Corresponding author: Email: jeenaprakash1@gmail.com;*

poultry were abundant because of the favourable climatic and poor standards of poultry husbandary [15,1]. Poultry diseases include viral, fungal and parasite pathogen that causes a major decrease in poultry production and thereby reduces economic benefits. This may affect the development of the poultry products [16].

2. METHODOLOGY

A total of 160 samples of indigenous birds were collected from Kulathupuzha, Kazhuthurutty and Thenmala in highland regions of Kollam District for a period of eight months (from October 2017 to May 2018). Gastrointestinal tract of the birds were collected from freshly slaughtered birds from local poultry shops, markets, household of rural areas of highland regions of Kollam District. The birds were divided into two groups: growers (9 to 16 wks) and adults (above 16 wks). Depending on the availability of rainfall, climatic condition could be divided into three seasons: summer (February to May) rainy (June to September) and winter (October to January). The intestines were dissected longitudinally and screened for the presence of helminths parasite [4]. Direct analysis of the trachea, small intestine and large intestine showed the presence of parasites. The worms were collected into sample tubes containing 70% ethanol for identification. Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel). The quality of the DNA isolated was checked using agarose gel electrophoresis. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). The main goal was to show the prevalence of helminths and the identification of helminths parasite to species level based on the DNA bar coding. The study showed the monthly abundance of different species of helminths in the gastrointestinal tract of *Gallus domesticus*. The prevalence of helminths was calculated using the equation [17].

Prevalence= (Number of chicken infected/ number of chicken examined) x100

3. RESULTS

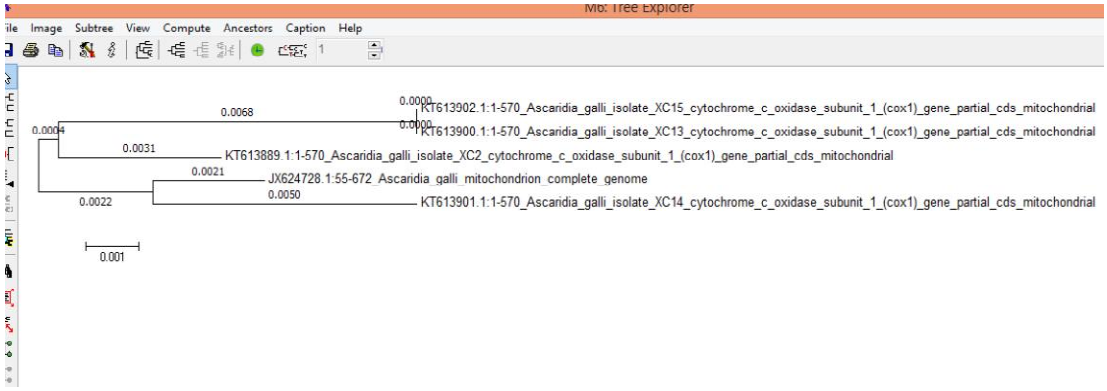
3.1 Sequences for Analysis

3.1.1 SEQUENCE: 1 >SR1089-1-COR_A02.ab1

GAGTGTTATAGAGAAAGAGAGATCCTCCGCC
CGGCAATCCCGATCTATGATGGTGTCCCTTTG
TTGACAGAGGGGACAGAAGTATTTAAATTAC
GATCAGTCAACAACATGGATAATAGCACCTG
CCAACACAGGAAGAGACAAAATTAACAAAAA
AACAGTAACAAACACAGTTCAAACAAACAAA
CTCATATGCTCCAAAGAAATAGAACTACTAC
GTAGATTCTTAGTAGTAGTCATAAAATTAATA
CCACCTAAAATAGAACTAATACCAGCACAAT
GAAGACTAAAAATAGCCAAATCCACCCTCCT
ACCAGGATGTCCACTAGTTCTCAAAGGAGGA
TAAACAGTTCAACTAGTACCACAACCACTATC
AACCAAAGTAGAACCCAAAATCAAAATTATA
GCAATAGGTAATAACCAAAAACTCAAATTAT
TCAAACGCGGAAACCTCATATCCGGAGCACC
TAACAGCAAAGGTAACATTCAATTACCAAAA
CCCCCAATTATAGTGGGCATCACTATAAAAA
AAATCATCAAAATAGCATGAGCAGTGATAAT
AGAATTATACAACTGCCCATCAGACAACAAC
AAACCAGGTTTAGCTAACTCTAAACGAATAA
TTAAAGACAAAGAACTACCAACCATACCAGA
CCAAATACCAAATAAAAAATACAAAGTCCCA
ATATCTTTATGTTTTGTTGACCCA

3.1.2 Sequence similarity

The sequence1 was subjected to similarity search by BLAST analysis; it was clearly shown that there was a 98% identity with the nucleotide sequence of *Ascardia galli.*

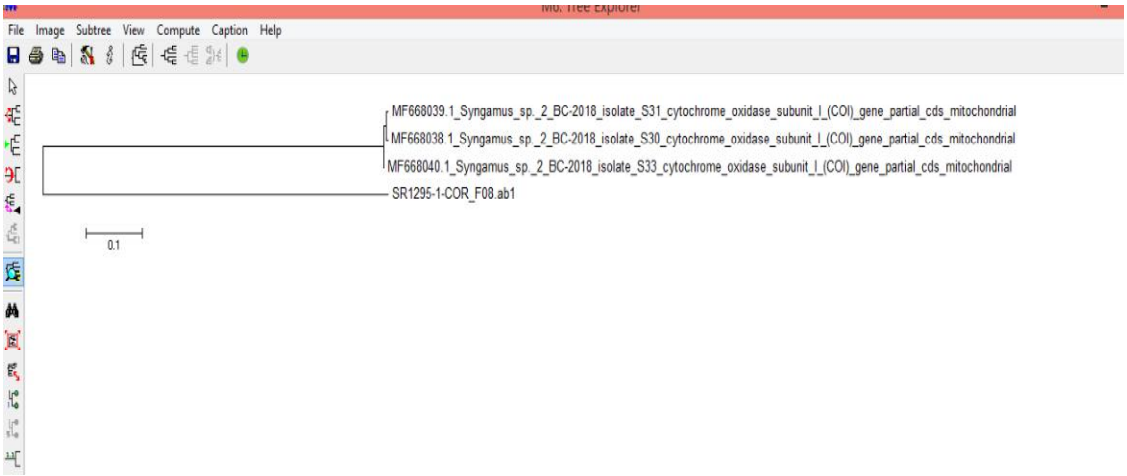


3.1.3 SEQUENCE: 2>SR1295-1-COR_F08.ab1

CAAGGGTGTTGGATTTGGGGTGGGAACACTC
CTCCTCCTGCGGGGTCGAAAAATGTGGTGTTA
TTGTTGCGGGCGGTAAGTATAATGGTTGTCCC
AGCTGCTAGGAGGGGTAAGGAGAGGAGTAGT
AGGATGGAAGTAATGAGGACGGATCAAACAA
ACTCGGGTGTTCGGTATTGTGACAGTGCGGGG
GGTTTTCTGTTGATGACGGATAAGATTAATAC
GACGGAAAATAGAACGCAGACCAGCACAGTG
TAAACTAAAGATAGCCAAATCAACACTCCTA
CCCGGATGCCCTAAAGTACTTAAGGGGGGAT
AAACCGTCCACCTAGTACCACAACCTATATCA
ACAAAACAAGAATCCAAAATCGAAAACATAG
CCGTAGGCGATAACCGAAAACTCAAATTATT
CAAACGAGGAAACCTCATATCAGGAGCCCCT
AATATTAAAGGCAACATCCAATTTCCAAAAC
CACCAATTATAGAAGGCATAACCATAAAAAA
AATCATTAAAATAGCAGGAGCAGTAATAATA
GAATTATACAACTGACCATTTCCCAACAAAAC
CCCAGGCTTAGACAACTCCAAACGAATAATT
AAAGACAAACTTCTCCCAATTATACCCGATCA
AAGACCAAAAAGAAAAATATAAACGTTCCAA
TATCTTTATGATTTTGTTGACCAGATTG

3.1.4 Sequence similarity

The sequence 2 was subjected to similarity search by Blast analysis. It was clearly demonstrated that the sequences had 89% identity with *Syngamus species*.



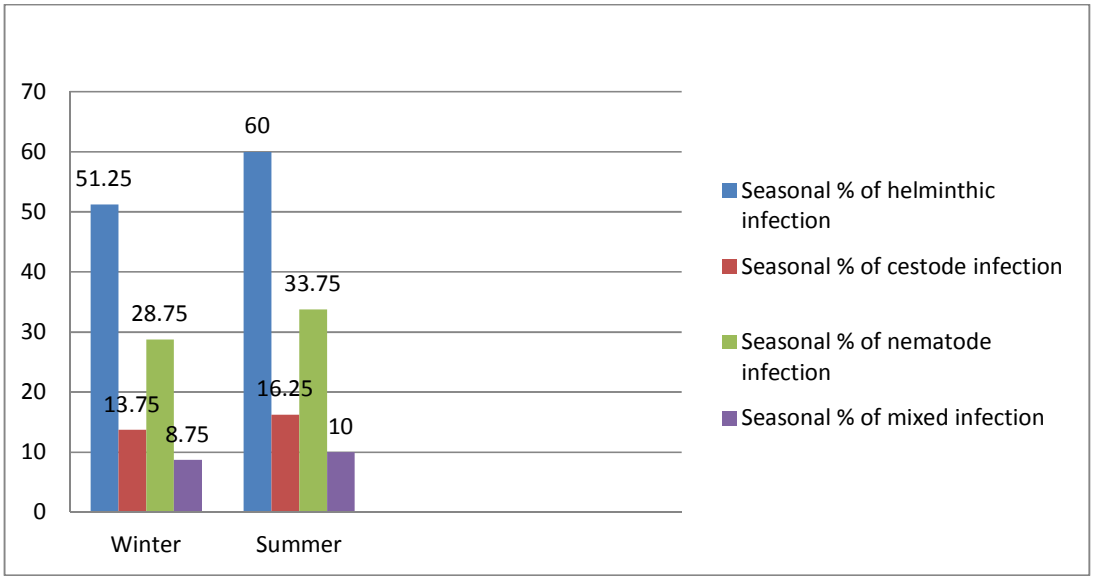


Fig. 1. Seasonal prevalence of helminth parasites in highland region of Kollam District

Table 1. Prevalence of gastro intestinal helminth parasite in *Gallus domesticus* in the Highland Region of Kollam District

Season	Month	No of birds examined	No & % of helminth infected birds	Seasonal % of helminthic infection	No & % of birds infected with cestode	Seasonal % of cestode infection	No & % of nematode infected birds	Seasonal % of nematode infection	No & % of birds with mixed infection	Seasonal % of mixed infection
winter	Oct	20	8 (40)	51.25	2 (10)	13.75	5(25)	28.75	1 (5)	8.75
	Nov	20	10 (50)		2 (10)		7(35)		1 (5)	
	Dec	20	12 (60)		4 (20)		6(30)		2 (10)	
	Jan	20	11(55)		3 (15)		5(25)		3 (15)	
summer	Feb	20	11(55)	60	4 (20)	16.25	6(30)	33.75	1 (5)	10
	March	20	12(60)		3 (15)		6(30)		3 (15)	
	April	20	13(65)		3 (15)		8(40)		2 (10)	
	May	20	12(60)		3 (15)		7(35)		2 (10)	
Total		160	89 (55.62)		24(15)		50(31.25)		15(9.37)	
mean				55.62		15		31.25		9.37

The major helminths parasite found was *Ascaridia species*, *Syngamous species*. Among these, the species most abundant was the species *Ascaridia galli*. The study, focused on the sequence analysis of the parasite and phylogenetic study of *Ascaridia species* and *Synganous species* based on the DNA bar coding. The nucleotide sequence data were aligned and saved the session as .fas file formats and then .mas file format. The sequence were subjected to phylogenetic tree construction using Mega 6 using distance based (Neighbour joining method) and character based approaches (Maximum parsimony).The tree with evolutionary distance .001 and xc13 and xc15 evolved from the same evolutionary period of *Ascaridia galli*. The tree with evolutionary distance 0.1evoled from the evolutionary period of *Syngamous species*.

In the high land, maximum prevalence of gastrointestinal helminth parasite was noted in the month of April (65%) and minimum in October (40%). Maximum Cestode infection was noted in the month of December and February (20%) and minimum in October and November (10%) whereas Nematode infection was maximum in the month of April (40%) and minimum in October and January (25%). Mixed infection (Cestode &Nematode) was maximum in the months of January and March (15%) and minimum in October, November and February (5%) (Table 1, Fig. 1).

Seasonal average of helminth parasites was maximum during the summer season (60%) and minimum during the winter (51.25%) whereas seasonal average of Cestode infection was maximum during summer season (16.25%) and minimum during winter (13.75%) (Table 1, Fig. 1). The seasonal average of Nematode infection was maximum during summer season (33.75%) and minimum during winter (28.75%) (Table 1, Fig. 1). Seasonal average of mixed infection was maximum during summer season (10%) and minimum during winter (8.75%) (Table 1, Fig. 1).

3.2 Prevalence of Helminth Parasite in the Highland Region of Kollam District in Relation to the Sex of the Birds

Sex-wise, the prevalence of helminth parasite showed that highest prevalence of helminth was noted in female than male during summer (60.09%, 58%) followed by winter (52.5%, 50%) (Fig. 2).

3.3 Age Wise Prevalence of Helminth Parasite in High Land

Age-wise, the prevalence of Helminth parasite showed that highest prevalence of helminth was noted in adult than grower during summer (68.75%, 46.87%) followed by winter (57.14%, 44.73%) (Fig. 3).

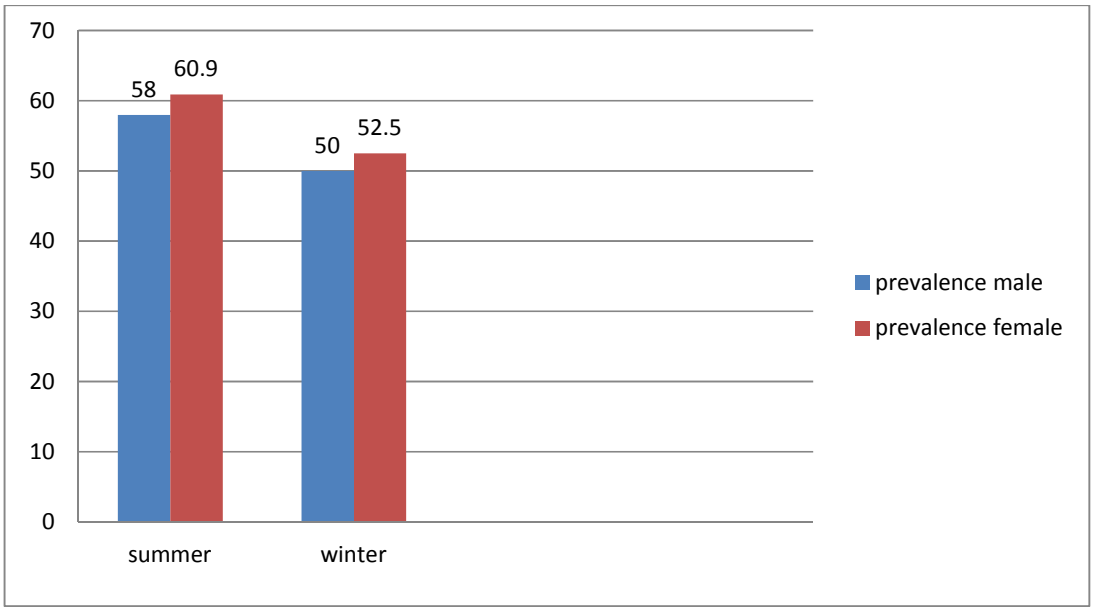


Fig. 2. Prevalence of helminth parasite in relation to the sex of the birds in high land region of Kollam District

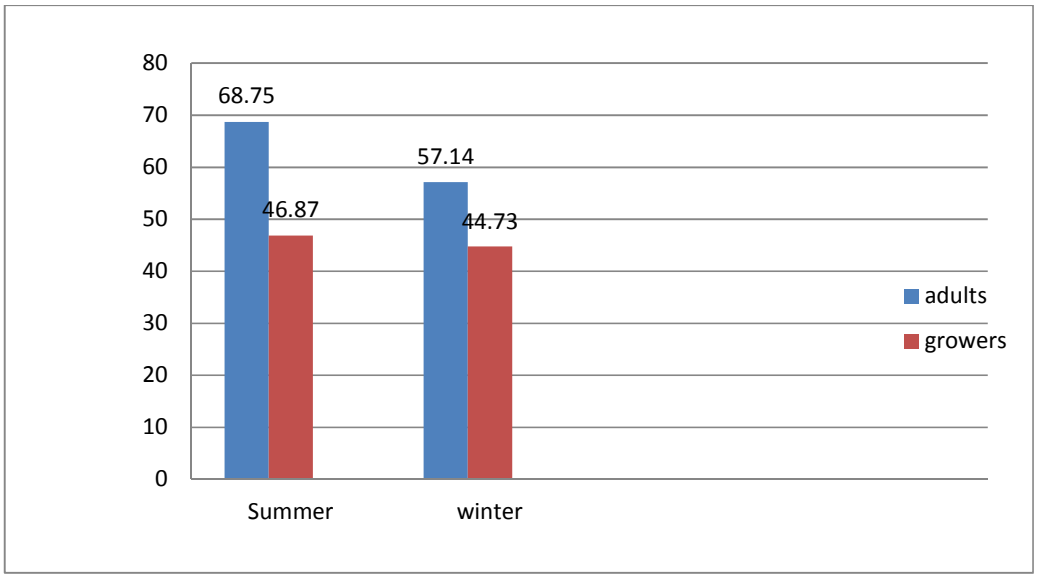


Fig. 3. Prevalence of helminth parasite in relation to age of birds in Kollam District

4. DISCUSSION AND CONCLUSION

In highlands, the percentage prevalence of helminth-infected male and female birds was highest during summer and lowest during winter (summer = 58%, winter 50% = for males and summer = 60.09%, winter = 52.5% for females) respectively. The present study showed that maximum prevalence of helminth was noted in females than the males. This may be due to longer reproductive lifespan of females as compared to male putting them under prolonged reproductive stress. And also the voracious feeding habits especially during egg production than males which remain largely selective which could have increased the risk of infection in females. Similar observation was done in Nigeria [9]. The study shows that the adult birds showed high prevalence of endoparasites. This may due to their gregarious habit and exposing to more intermediate host than chick [2,6]. Similar report was observed in Marathwada region, India [10] showing that in relation to season, the highest prevalence was recorded during summer (83.96%) followed by the rainy season (77.66%) and lowest during winter (64.81%). Similar report in Turkey showed highest seasonal incidence of helminthic infection during summer (91.5%), followed by spring (78.43%), then by autumn (68.62%) and lastly by winter (46.80%) [5]. Studies also showed that the highest prevalence of helminth infection was recorded during summer (91.5%) and the lowest was recorded during winter (46.80%) [8]. Studies also observed high prevalence of endoparasite during summer as compared to winter and rainy seasons in free ranging birds [12,11,14]. In comparing nematode and cestode infections, the nematode were found to be dominant over the cestode species by observation on the incidence of helminths in Deshi fowls in Punjab [3]. Many insects that may act as vectors for helminth infection are also favoured by high temperature and to some extent of humidity [13]. The intensity of prevalence of helminth infection by the parasites was different in different regions and some differences related to the environmental factors that shows the availability of intermediate hosts [10]. This study showed that the prevalence of parasite helminthes *Ascaridia* species was most commonly seen in *Gallus domesticus*. Based on the DNA bar coding of the sample, the sequence 1 for similarity search by BLAST analysis; it was clearly shown that 98% identity was with the nucleotide sequence of *Ascaridia gallium* (the most prevalent pathogenic species in *Gallus domesticus* Linnaeus (1758)) [7]. Considering the DNA bar coding of sequence 2 for similarity search by BLAST analysis, it was clearly showed that 89% identity was with *Syngamus species*. The parasites causes ascaridiasis and syngamiasis in chicken. Therefore necessary measures should be implemented to control parasitic helminths in chicken. Continued improvements in the management, sanitation practices and further studies on parasitic helminths in chicken should be undertaken for better quality of poultry products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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