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Prevalence of Gastrointestinal Helminthic Infections in Black Bucks (*Antilope cervicapra*) of Tal Chhapar Sanctuary of Rajasthan

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ABSTRACT

Background: Blackbuck is the most elegant member of the antelope family has long been associated with Indian culture. Wildlife parasitic diseases represent an important field of investigation as they may have a significant impact on wild animal health and are responsible for one-third of total losses due to all animal diseases. Adequate information on epidemiology of helminthic infections is a crucial requirement for the sustainable control of GIH in black bucks in near future.

Methods: A total of 632 faecal samples were collected from Tal-Chhapar Sanctuary of Rajasthan during summer, rainy and winter season from November 2018 to September 2019 and examined qualitatively by faecal floatation and sedimentation techniques for helminth eggs and quantitatively by modified McMaster egg counting technique.

Result: Coprological examination of samples revealed an overall prevalence of 72.46% for gastrointestinal helminths (GIH) with a mixed infection of 38.92%. Among different helminths recorded with their respective prevalences (%) were, Strongyle (56.80%), *Strongyloides* sp. (40.98%), *Trichuris* sp. (8.22%), *Marshallagia* sp. (6.64%) and *Ascaris* sp. (5.22%). Quantitative analysis revealed egg per gram of faeces ranging from 200-1200 and 200-900 with an average of 561.11±67.74 and 469.23±62.50 for strongyle and *Strongyloides* sp., respectively. Statistical analysis using multivariate binary logistic regression model revealed highly significant difference (P<0.01) in the prevalence of GIH infection among different seasons. The coproculture study revealed *Bunostomum* sp. (42%) as the major contributor of strongyle nematode population, followed by *Haemonchus* sp. (31%), *Trichostrongylus* sp. (26%) and *Strongyloides* sp. (1%).

Key words: *Antilope cervicapra*, Gastrointestinal helminths, Prevalence, Rajasthan, Tal chhapar sanctuary, Wildlife.

INTRODUCTION

The blackbucks (*Antilope cervicapra*), popularly known as the Indian antelope are indigenous to the Indian subcontinent where their population has decreased by excessive hunting and loss of their natural habitat. The blackbuck (*Antilope cervicapra*) is gregarious and social animal with herds which belongs to the genus *Antilope* and is classified under the family bovidae and subfamily antelopinae. The blackbuck has recently moved from the "Near threatened" to "Least Concern" in Red Data Book of IUCN (IUCN, 2017) and categorized in Appendix III of CITES. Among 13 states of India in which Blackbuck is found, Rajasthan is known for its rich and diversified range of wild animals and comprises of major portion of blackbuck population of the country (FSI, 2015).

In recent years, apart from poaching and destruction of habitats, health related issues have become a significant threat to wildlife. Wild animals suffer from a variety of infectious and non-infectious diseases, particularly that of parasitic origin (Akhter and Arshad, 2006; Lama *et al.*, 2015). In nature, wild animals live on large areas and have consequently a low genetic resistance against parasitic infections because of low exposure (Muoria *et al.*, 2005). Wildlife parasitic diseases represent an important field of investigation as they may have a significant impact on wild animal health and may also have public health concern (Liatis *et al.,* 2017). Blackbucks are susceptible to various kinds of parasitic infections like coccidiosis, paramphistomosis, fasciolosis, schistosomosis, taeniosis and nematodosis (Thornton *et al.*, 1973). Parasitic infections cause nearly one-third of total losses due to all animal diseases (Anonymous, 1990) and overlooked as the majority of the infected animals show a number of little obvious clinical signs during their productive life and their effects are gradual and chronic (Raza *et al.*, 2007; Gelot *et al.,* 2016).

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Information on parasitic infections of wild animals is limited due to lack of systematic investigations (Varadharajan and Kandasamy, 2000). A number of studies for gastrointestinal parasitic prevalence in black bucks have been carried out in India by Thawait *et al*. (2014), Mir *et al*. (2016) and Das *et al*. (2018) in Chhattisgarh, Punjab and Odisha, respectively. Information on parasitic infections of blackbucks is meager in Rajasthan as only a single study for gastrointestinal parasites of blackbucks has been reported by Pilania *et al*. (2014). No such study in Tal-Chhapar sanctuary has been reported so far.

Adequate information on epidemiology of helminthic infections is a pivotal requirement for the sustainable control of GIH in black bucks. Keeping in view these facts, the present study has been aimed to map the prevalence rate, severity and types of helminthic infections in black bucks of Tal-Chappar sanctuary.

MATERIALS AND METHODS

The study area comprises of Tal-Chhapar sanctuary, which is located in Churu district of north-western Rajasthan in India and is spread over 7.19 Sq. Km area. Tal-Chhapar sanctuary comes under principal arid zone of the country and is characterized by large variation in temperature which reaches up to 48°C in June and minimum temperature falls below 4°C in December-January. The area is characterized by stormy southwest winds and frequent dust storms with an average rainfall of 300 mm (D.O.A., Govt. of Rajasthan, www.agriculture.rajasthan.gov.in).

The status of prevalence rate of infection, the expected prevalence of 50% with confidence limits of 95% and a desired absolute precision of 5% was studied by collecting maximum number of representative samples (Thrusfield, 2005). The number of samples thus calculated was adjusted for finite population and was correlated with 632 samples collected randomly from blackbuck population collected from Tal Chhapar Sanctuary of Rajasthan during summer, rainy and winter season from November 2018 to September 2019. The samples were placed in sterile polythene bags and labelled carefully indicating the host's detail, location and month of collection, kept in a cool transport box and brought to the Laboratory for further examination.

Faecal samples were qualitatively examined by faecal floatation and sedimentation techniques (Solusby, 1965) for helminthic eggs and quantitatively by modified McMaster egg counting technique (Coles *et al*., 2006). Coproculture study was also performed to harvest and identify infective strongyle larvae (Solusby, 1965). Cultured larvae were harvested using Baermann's apparatus and were identified as per the key provided by Wyk and Mayhew (2013).

All data analyses were performed by using SPSS 20.0 software for Windows by applying Chi square (χ^2) test and variables with significant association at $P \leq 0.05$ (two-side) were subjected to the multivariate Binary logistic regression model.

RESULTS AND DISCUSSION

The overall prevalence for gastrointestinal helminths in the Blackbucks was recorded 72.46% during current study which is in compliance to the reports from many other Indian states of Punjab (Mir *et al*., 2016) and Odisha (Das *et al*., 2018) and from around the world including Latvia (Ruta *et al*., 2009) and Bangladesh (Barmon *et al*., 2014; Rahman *et al*., 2014). The high prevalence may be due to favorable climatic conditions, constant exposure of infestation and availability of infective stage larvae on the grazing ground by animals (Lama *et al*., 2015; Opara *et al*., 2010).

In contrast, lower prevalence have been reported in various wild and captive ruminants including Blackbucks from Aurangabad, Maharashtra (Khan *et al*., 2014), from Raipur, Chhattisgarh (Thawait *et al*., 2014) and from Thrissur, Kerala (Jaya and Aja, 2018) whereas, a higher prevalence was recorded by Gupta *et al*. (2011) from Jabalpur, M.P., by Pilania *et al*. (2014) from Bikaner, Rajasthan and by Nayak *et al*. (2018) from Bhubaneswar, Odisha. However, the variations regarding prevalence in various studies can most likely be attributed to difference in number and distribution of animals and variation in topography and climatic factors (Das *et al*., 2018).

Strongyle infection (56.80%) was identified as the most prevalent nematode infection during present study followed by *Strongyloides* sp. (40.98%), *Trichuris* sp. (8.22%), *Marshallagia* sp. (6.64%) and *Ascaris* sp. (5.52%) in the decreasing order of prevalence with complete details in Table 1 and Fig 1. The various helminth parasites reported from Blackbucks of Tal-Chappar sanctuary of Rajasthan in the present study are in accordance with the previous findings of Pilania *et al*. (2014) from Rajasthan state and Singh *et al*. (2006), Meshram *et al*. (2008), Sahoo *et al*. (2009), Mir *et al*. (2016) and Das *et al*. (2018) from various states of India. It was observed that Blackbucks have been infected highly with nematode parasites in comparison to trematode and cestode parasites (Pilania *et al*., 2014; Mir *et al*., 2016) which is consistent to the findings of this study.

Seasonal dynamics of GIH infections revealed a highly significant difference ($p<0.01$) among seasons with highest prevalence in rainy season (80.71%) which is congruent with the reports of Kumar *et al*. (2009), Sahoo *et al*. (2009), Mahali *et al*. (2010) and Das *et al*. (2018) from different parts of India. No season targeted study has been reported from the state of Rajasthan. The highest prevalence in rainy season might be due to more suitable environmental conditions in terms of humidity and temperature for the development of pre-parasitic stages of most of parasitic nematodes, whereas cold and dry climate have destructive effects on the development of the helminthic stages and allow fewer pre infective larvae to reach the infective stage (Soulsby, 1982). On the other hand, subsequent occurrence of gastrointestinal helminth infections was observed in summer (78.26%) followed by winter season (57.42%) during the present study which showed consistency with the observation of Kumar *et al*. (2009), Sahoo *et al*. (2009),

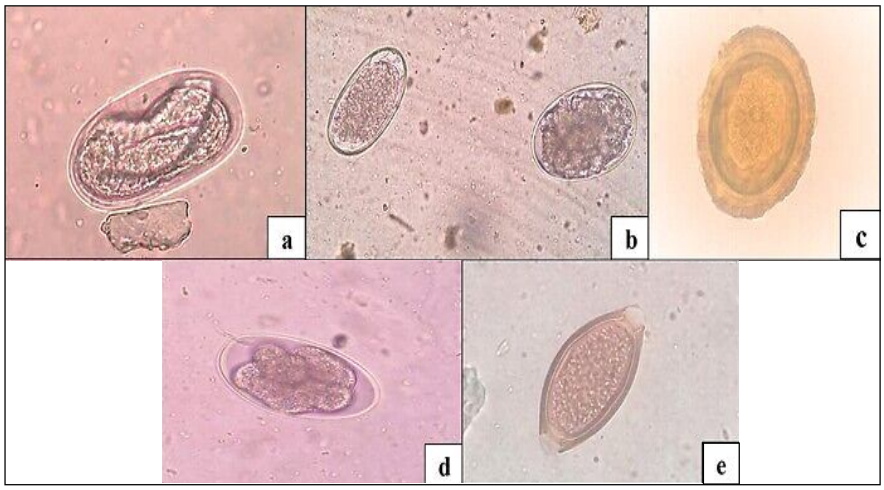

Fig 1: Micrographs of various Gastrointestinal Helminth eggs (40X).
(a) *Strongyloides* sp. (b) Strongyle type (c) Ascaris sp. (d) *Marshallgia* sp. (e) *Trichuris* sp.

Table 1: Overall and season wise prevalence of gastrointestinal helminth infections in black bucks of Tal Chhapar Sanctuary, Rajasthan.

		Examined	Infected	Mixed	Gastrointestinal Helminths				
					Strongyle	*Strongyloides*	*Trichuris*	*Ascaris sp.*	*Marshallagia* sp.
Season	Winter	202	116 (57.42)	57 (28.21)	66 (32.67)	76 (37.62)	24 (11.88)	6 (2.97)	6 (2.97)
	Summer	207	162 (78.26)	82 (39.61)	133 (64.25)	72 (34.78)	20 (9.66)	21 (10.14)	7 (3.38)
	Rainy	223	180 (80.71)	107 (47.98)	160 (71.74)	111 (49.77)	8 (3.58)	6 (2.69)	29 (13.00)
χ^2 value		-	33.996**	17.47**	72.91**	11.36**	10.49**	15.09**	22.48**
Overall		632	458 (72.46)	246 (38.92)	359 (56.80)	259 (40.98)	52 (8.22)	33 (5.22)	42 (6.64)

Note: Figures in parentheses indicate percentage, *= significant, **= highly significant.

Mahali *et al.* (2010) in various states of India and Barmon *et al.* (2014) from Bangladesh. This study revealed that heavy rainfall and high relative humidity predisposed the animals to heavy nematode infection. Statistical analysis using multivariate binary logistic regression analysis revealed a positive association in summer and rainy season *i.e.* odd ratio of infection increased by 1.512 in summer and 1.832 in rainy season as compared to winter with complete details in Table 2.

Among the various helminth infections, highest incidence of strongyle (71.74%) and *Strongyloides* sp. (49.77%) was recorded during rainy season in the present study. These observations are similar to the reports Singh *et al.* (2009) and Mir *et al.* (2016). Prevalence of *Marshallagia* sp. was also reported highest in monsoon which is congruent to the incidences reported by Eslami *et al.* (1980) and Sharhuu and Sharkhuu (2004). The availability of fresh grass during rainy season for grazing of Blackbucks in Tal-Chappar sanctuary of Rajasthan probably may be a decisive factor for risk of helminth infections. The present study revealed the highest incidence of *Trichuris* sp. (11.88%) during winter season whereas highest incidence of *Ascaris* sp. (10.14%) during summer season which is in close approximation to the findings of Hussain *et al.* (2002) and Meshram *et al.* (2008) from Nagpur and Western Vidarbha region of Maharashtra, respectively.

Table 2: Multivariate binary logistic regression for gastrointestinal helminth infections in black bucks.

Parameter		Logistic regression coefficient (B)	S.E.	Wald test	Odd ratio
Season	Winter	-	-	32.863	
	Rainy	0.414	0.110	14.194	1.512
	Summer	0.606	0.106	32.589	1.832
Constant		-1.542	0.083	348.67	0.214

Note: S.E.= Standard Error.

Quantification of the infection by means of eggs per gram (epg) revealed mild to severe infection of *Trichuris* sp., *Strongyloides* sp. and strongyle with an average of 316.67±87.22 (100-600), 469.23±62.50 (200-900) and 561.11±67.74 (200-1200) epg counts, respectively with highest intensities during rainy season (Table 3). Similar intensity of strongyle infection was reported by Singh *et al.* (2009) and Chaudhary and Maharjan (2017), however they recorded a lower intensity for *Strongyloides* sp. in contrast to the findings of present study. The variations may be due to the differences in climate-ecology, management, sample size and time of sampling of the respective study areas. Sufficient rainfall and moisture during wet season favor the survival of infective larvae in pasture and higher probability of uptake of the infective larvae leading to higher prevalence rate and higher egg counts (Kuchai *et al.*, 2010).

Table 3: Intensity of gastrointestinal helminth infections in black bucks (Mean±S.E.).

Parameter		Strongyle	*Strongyloides* sp.
Winter	Range	200-800	200-600
	Mean±S.E.	500±96.98	375±85.39
Summer	Range	200-900	200-700
	Mean±S.E.	500±106.55	425±110.86
Rainy	Range	200-1200	200-900
	Mean±S.E.	683.33±147.57	580±116.07
Total	Range	200-1200	200-900
	Mean±S.E.	561.11±67.74	469.23±62.50

The coproculture studies revealed *Bunostomum* sp. (38%) as the major contributor to the nematode population followed by *Haemonchus* sp. (24%), *Trichostrongylus* sp. (22%) and *Strongyloides* sp. (16%) in decreasing order of prevalence. The findings of present study are in close agreement with the findings of Fathima *et al*. (2019) and unlike to the findings of Cisek *et al*. (2003), Meshram *et al*. (2008) and Farooq *et al*. (2012).

Seasonal coproculture analysis revealed *Bunostomum* sp. larvae predominance during rainy season whereas *Haemonchus* sp. was most dominant during summer season. Higher rate of infection in rainy months may also be attributed to suitable molarity of salt present in soil which is an important factor for ecdysis (Soulsby, 1982). Development and survival of pre-helminthic stages of *Haemonchus* sp. are highly influenced by hot and humid weather (Kates, 1950), total monthly rainfall (more than 50 mm) and mean monthly maximum temperature (more than 18.3°C) (Gordon,1953) which was approximate to the meteorological conditions of the study area during the present study (D.O.A., Govt. of Rajasthan, www.agriculture.rajasthan.gov.in).

CONCLUSION

Present study represents a comprehensive report on gastrointestinal helminth infection in black bucks of Tal Chhapar Sanctuary, Churu, Rajasthan and the data generated could be of immense help in formulation of effective strategies for GIH control in order to upgrade the health and conservation status of black buck population.

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Conflict of interest

We declare that we have no conflict of interest.

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