A field survey on the status of gastrointestinal helminth parasites in hangul (*Cervus elaphus hanglu*) in Dachigam National Park of Kashmir

Bashir A. Lone · M. Z. Chishti · Fayaz Ahmad · Hidayatullah Tak · Suhaib A. Bandh · Abida Khan

Received: 25 February 2014 / Accepted: 8 September 2014 / Published online: 30 October 2014
© Indian Society for Parasitology 2014

Abstract One year crosssectional survey was carried out to determine and describe the prevalence and intensity of gastrointestinal parasite infections in hangul (*Cervus elaphus hanglu*) in Dachigam National Park of Kashmir through faecal examinations. Out of 153 faecal samples examined, 82 (53.59 %) were found infected with GIT helminthes. In present study seven helminth species were found, including five nematode [*Haemonchus contortus* (55.39 %), *Trichuris ovis* (39.75 %), *Dictyocaulus viviparus* (28.4.00 %), *Oesophogostomum circumcincta* (13.7 %) and *Chabertia ovina* (4.02 %)] one trematode [*Fasciola hepatica* (17.3 %)] and one cestode species [*Moneizia expansa* (6.05 %)]. Based on the severity of infection 81.7 % of hangul positive samples

were severely infected (epg > 1,500), 8.3 % heavily infected (epg = 1,100–1,500), 3.8 % moderately infected (epg = 800–1,000) and 7.2 % mildly infected (epg = 500). Season, sex and age were the factors that influenced the epidemiological prevalence of GIT helminths in hangul in the present study. The maximum helminth infection was observed in summer season and lowest in winter ($P = 0.003$). Lower age groups were more infected than adult animals ($P > 0.05$). Prevalence was higher in males than females ($P > 0.05$). The present study will initially be of great significance to add to existing knowledge of the epidemiology of GIT helminth of hangul which is the pioneering study on this animal in the valley and the findings will be quite helpful to devise the appropriate control and prophylactic strategies for GIT helminthiasis of hangul in the Dachigam national park.

Keywords Epidemiology · Helminth · Prevalence · Sex · Season · Hangul · Dachigam national park

B. A. Lone (✉) · M. Z. Chishti
Parasitology Research Lab., Centre of Research for
Development, University of Kashmir, Srinagar 190006, India
e-mail: bashir.lone@gamil.com

M. Z. Chishti
e-mail: drchishti@rediffmail.com

F. Ahmad · H. Tak · A. Khan
Department of Zoology, University of Kashmir,
Srinagar 190006, India
e-mail: rajafayazali@yahoo.com

H. Tak
e-mail: drhidayattak@yahoo.com

A. Khan
e-mail: khan.renu2@gmail.com

S. A. Bandh
Microbiology Research Lab., Centre of Research for
Development, University of Kashmir, Srinagar 190006, India
e-mail: suhaibbandh@gmail.com

Introduction

Hangul, Kashmiri stag being the state animal is a subspecies of European Red deer. Although more than 150 species of deer are recognized globally, Hangul is the only surviving race of the Red deer family of Europe in the subcontinent as the Shou (*Cervus elaphus Wallichi*) of Bhutan is now considered extinct. Large forest cover has been lost because of anthropogenic disturbance and continues to decline at a rate of 0.7 % annually ([FAO] Food and Agriculture Organization 1999) resultant habitat loss of hangul is endangered.

Although parasite infections are common in nature and low intensity infections are often asymptomatic (Anderson and May 1979), anthropogenic change may result in a loss

of stability associated with altered transmission rates, host range, and virulence (Patz et al. 2000). Within this context, baseline data on patterns of parasitic infections in wild deer populations are critical to provide an index of population health and to begin to assess and manage disease risks. Wildlife was found to be susceptible to gastrointestinal parasites of livestock. In wild animals which are kept in captivity or live in limited spaces, because of overcrowding, the chances of parasitic infections are usually high (Halvorsen 1986).

Although many studies have documented the gastrointestinal parasites of ruminants of Kashmir (Dhar et al. 1982; Lone et al. 2012; Tariq et al. 2008a, b, c) but the gastrointestinal parasites of other Kashmiri wild taxa remain poorly discovered. The present study identifies and quantifies the gastrointestinal helminth parasites for the Hangul, Kashmiri stag (*C. elaphus hanglu*).

Materials and methods

One year crosssectional faecal survey was carried out from January 2010 to December 2010, on hangul. A total of 153 fecal samples were examined from free-ranging hangul at forested sites in Dachigam National Park located 22 km from Srinagar, Jammu and Kashmir (Dachigam ranging from 5,500 to 14,000 ft above mean sea level, 141 km² in area and 34°8'14"N 75°2'16"E) The monthly collection of the samples from various feeding sites of the Dachigam National park was carried out in order to ensure the seasonal prevalence from both the sexes.

Study area

Kashmir valley is a beautiful part of the temperate Himalayan region, which is an important agro climatic region of

India. It lies between 33°–20' and 34°–54' North latitude and 73°–55' and 75°–35' East longitude covering an area of 15,948 sq. km. Topographically, it is a deep elliptical bowl shaped valley bounded by lofty mountains of the Pir Panjal range in the south and south west and the Great Himalayan range in the north and east, with 64 % of the area being mountainous (Fig. 1) (Dar et al. 2002). Forages and grasses are the major source of feed for hangul sustenance in this national park. These are produced on pastures, meadows and on open lands of the park. Hangul graze throughout the year except winter when the whole park is covered with snow, feed consisting of dry salix leaves, dry grasses, and other dried plants.

Climate

The climate is a typical warm temperate. The temperature ranges from an average daily maximum of 30 °C and minimum of 15 °C in June–July to an average daily maximum of 4 °C and minimum of −9 °C in January. The absolute humidity ranges from 50 to 80 % throughout the year and drops to about 55 % at night during the winter and 64 % during the summer. The whole valley is covered with snow in winter. On the basis of temperature and precipitation, four seasons in a year recognized in Kashmir valley are: winter (December–February); spring (March–May); summer (June–August); autumn (September–November) (Dar et al. 2002). The occurrence of helminthiasis in an area is influenced by a multifactorial system which comprises hosts, parasite and environmental effects (Lone et al. 2011) (Fig. 2).

Faecal examination

Samples were collected immediately after defecation to avoid contamination and examined macroscopically for

Fig. 1 Dachigam National Park (Jammu and Kashmir state gazette 2010)

Fig. 2 Monthly mean of temperature, absolute humidity and rainfall during the year 2010 in Dachigam National Park

adult nematodes and tapeworm proglottids. Samples were stored individually in 5.0 ml sterile vials in 10 % neutral formalin solution. Preserved samples were transported to the Parasitology Research Laboratory, Centre of Research for Development, University of Kashmir where faecal samples were microscopically examined for helminth eggs, larvae and protozoan cysts using concentration methods by Zinc sulphate flotation, fecal sedimentation and Modified McMaster techniques (Sloss et al. 1994; Urquhart et al. 1996). Parasites were identified on the basis of egg color, shape, contents, and size (Soulsby 1982). Levels of worm infection were extrapolated from infection severity index defined by Soulsby (1982) where hangul were said to have mild, moderate, heavy and severe nematode infestation if their fecal egg counts are less than 500, 800–1,000, 1,100–1,500 and more than 1,500, respectively.

Faecal cultures were done for samples with total mean egg counts of greater than 10,000. Coprocultures were used to match parasite eggs to larvae for positive identification of nematodes ([MAFF] Ministry of Agriculture Fisheries and Food 1979). Our ability to identify most parasite species from host fecal examination, even with cultured larvae, is limited. Consequently, we present the majority of our findings at the level of family or genus.

Data analysis

Percentages with their respective mean $\pm$ SEM (standard error of the mean) and 95 % confidence intervals were employed to calculate the prevalence of helminth parasites. Chisquare test was employed to measure association between the parasitism and season, age and sex. Eggs were estimated as number of eggs per gram of faeces with their respective median and percentile. The data was analyzed using Statistical packages MINITAB software version 13.2 (Minitab 2002) and SPSS-17 for windows. In all the

analysis, confidence level was held at 95 % and $P < 0.05$ for significance.

Results and discussion

Overall prevalence

An epidemiological investigation on GIT helminths was conducted among naturally infected hangul from the Dachigam national park in Kashmir valley. This endeavour revealed a spectrum of GIT helminth in naturally infected hangul in Kashmir valley. The parasites in decreasing order of prevalence (%) were *Haemonchus contortus* (55.39 %), *Trichuris ovis* (39.75 %), *Dictyocaulus viviparus* (28.400 %), *Fasciola hepatica* (17.3 %), *Oesophogostomum circumcincta* (13.7 %), *Moneizia expansa* (6.05 %) and *Chabertia ovina* (4.02 %) and their egg (eggs per gram) of faeces were shown in Fig. 3. Mixed infections were detected in 54.8 % of the hangul. Based on the severity index defined by Soulsby (1982), 81.7 % of hangul sampled were severely infected, 8.3 % heavily infected, 3.8 % moderately infected and 7.2 % mildly infected. The maintenance of high infection rate of parasitic helminths in the park might be associated with lack of any parasitic helminths intervention program and the management system in the national park where many small ruminants were allowed to graze together on small plots of land throughout the year which facilitates contamination of grazing grounds.

Out of 153 faecal samples examined, 82 (53.59 %) were found infected with GIT helminths. The overall intensity of infection was very low as was obvious from the low/moderate faecal egg counts in different seasons. A great variation in temperature occurs and a grazing break during winter season disrupts the continuous cycle of infection

Fig. 3 Box plot model for eggs per gram of faeces of helminth parasites

between host and pasture, due to which transmission of parasites does not occur throughout the year. The pastures of Dachigam National Park are, therefore, contaminated during the summer seasons only by grazing of other ruminants and this results higher prevalence of infection during these months.

Prevalence of helminth parasites recorded in the current study was lower than that reported earlier (81.17 %) by Pandit et al. (2003) and (61.64 %) Tariq et al. (2008a) in small ruminants of Kashmir. (Nashiruddullah et al. 2005, 2007) also observed the *D. viviparus* from the lungs of hangul they also described histopathological lesions in natural infection of *D. viviparus* in Kashmiri stag or hangul (*C. elaphus hangalu*). *H. contortus* the tropical and sub-tropical parasite was found to be highly prevalent in Kashmir valley—the temperate climatic zone of Indian subcontinent. The increase in temperature due to global climatic alterations can be one of the possible factors behind their occurrence in this temperate climatic zone. Earlier, Dhar et al. (1982) recovered *H. contortus*, *T. axei*, *T. colubriformis*, *B. trigonocephalum*, *C. ovina*, *N. spathiger*, *Skrjabinema ovis* and *T. ovis* from sheep in Kashmir. Tavassoli et al. (2010) reported rate of infection for Strongylid, *Marshallagia*, *Trichuris* eggs, and lung worm larvae as 8 (19.5 %), 12 (29.5 %), 17 (41.5 %) and 14 (34.1 %), respectively in wild sheep of Iran.

Influence of seasons on the prevalence of helminth parasites

The data pooled for seasonal estimation of helminth infection revealed definite seasonal patterns of infection in hangul over the period of one year with highest infection in summer and lowest in winter (Table 1). The faecal egg counts were

affected by months of the year and were quite variable in different seasons with significant differences ($P = 0.03$). Pearson's correlation coefficient ($r = +0.947$) calculated between different seasons with helminth infection revealed definite season wise association towards infection as the value always approached +1.

The highest prevalence (84.21 %) and high faecal egg counts (1858.3 ± 298.15 %) were found in summer season. From December onwards till January there was a great reduction in faecal egg counts. The low infection reported in the winter season could be attributed to complete absence of grazing in winter and also important is low temperature which helps in hypobiosis of nematodes in host and unavailability of intermediate hosts (Ogunsuri and Eysker 1979; Gibbs 1986). The observations are in agreement with earlier workers (Vlasoff et al. 2001; Shahadat et al. 2003; Tariq et al. 2008c; Lone et al. 2012) who reported low winter prevalence of GIT helminths in other ruminants. The presence of sufficient moisture and optimum temperature conditions during the rainy seasons (spring and summer) favoured the survival of infective larvae in the pasture and higher probability of uptake of the infective larvae leading to higher prevalence rate in the summer season. In line with this seasonal trend are the observations of various authors (Vlasoff et al. 2001; Khajuria and Kapoor 2003; Lateef et al. 2005; Tariq et al., 2008c). The present observations suggest that grazing animals are prone to get high level of GIT helminth infection during the rainy months and soon after. The subsequent infection has detrimental effect on the hosts because of the poor nutritional status of the animals in snowy winter season. Thus, climatic conditions have a great role to play in the onset of helminthic infections in hangul. It is, therefore, important to work further on this important issue.

Table 1 Influence of seasons on the prevalence of gastrointestinal helminth infection in hangul over the period of 2 years in Kashmir valley

Season	Examined	Infected (%)	Mean epg (range)
Spring	25	10 (40.00)	1253.3 ± 189.24 (648.0)
Summer	38	32 (84.21)	1858.3 ± 298.15 (1014.0)
Autumn	42	26 (61.90)	1683.3 ± 162.57 (532.0)
Winter	48	14 (29.16)	160.0 ± 36.89 (122.0)
Total	153	82 (53.59)	$P = 0.03$ $r = + 0.9471$

Influence of sex on the prevalence of helminth parasites

Sex wise investigation was found to be of no significant influence on the prevalence of helminthosis in hangul, however, small sample size precluded comparisons of prevalence (Table 2). The males were slightly more infected with nematodes than females; however the observations were not statistically significant ($P = 0.82$) but Pearson's coefficient was ($r = + 0.999$) between infected male and female was almost +1. The influence of sex on the susceptibility of animals to infections could be attributed to genetic predisposition and differential susceptibility owing to hormonal effects. The present gender wise observations are in agreement with Gulland and Fox (1992) who reported prevalence of nematode infection of sheep higher ($P < 0.05$) in rams than in ewes. Although no difference in prevalence of infection was apparent between male and female hangul for shared parasite species.

Influence of age on the prevalence of helminth parasites

Age wise epidemiological observations revealed highest prevalence in lower age groups of hangul (Table 2). Gastrointestinal helminth eggs were found in 75 % of the fawns, and 49.61 % of the adult hangul. The mean egg counts per gram of faeces in fawns, and adult hangul were 1892.6 ± 182.40 [1709.6–2074.4] and 1341.8 ± 86.80 [1,255–1428.6] respectively. With the increase in age the infection level decreased ($P > 0.05$). The lower age groups of animals found to be infected more is because of the low

resistance in young animals. The low level of parasitism reported in adult animals is due to the development of significant immunity, which is initially low but increases with the intensity and duration of exposure of infection. Age is an important factor in the onset of infection in host body. Age was found to have a significant influence on the level of risk of helminth infections in hangul with high prevalence in young hangul than adults (Magona and Musisi, 2002). Our observations are also in accordance with the findings of other authors who have worked on other ruminants (Vlasoff et al. 2001; Vanimisetti et al. 2004; Lateef et al. 2005). This may reflect energy and nutrient stress associated with producing and raising infants, which may result in an increased susceptibility to infection (Gulland and Fox 1992).

It was noticeable that all helminth parasites encountered during this survey were also found in other ruminants despite the habitat diversity and extensive range and grazing area. However, all being ungulates are sharing the same grazing and watering sites, particularly during the summer season; this might predispose wildlife to infestation with all these parasites and explain their high susceptibility to these parasites. However, experimental studies with the hangul cannot be simulated owing to the highly endangered and scheduled status and elusive nature of the hangul in the wild.

In conclusion, the present study indicated that quantitative FEC's are indispensable for monitoring levels of parasitic infestations and pinpointing problem herds. The nematode species recorded in this study are known to be of pathogenic importance. Our study contributes baseline data on the patterns of parasitic infection in wild hangul, providing a first step toward an index of population health and disease risk assessment for conservation and management plans of threatened hangul populations. The helminth fauna of hangul was similar to other ruminants their access to the national park should be checked to prevent pasture contamination. Gastrointestinal parasite classification by fecal analysis is weak by its very nature. However, it is the only responsible method to approach threatened species. Future studies using molecular analysis and opportunistic necropsies are needed to improve our classification of the gastrointestinal parasites of hangul, as well as to improve

Table 2 Influence of sex and age on the relative risk of hangul to gastrointestinal nematode infection during the period of 2 years in Kashmir valley

Factor	Level	Examined	Infected (%)	Mean epg (range)	P value
Sex	Male	30	17 (56.66)	1428.5 ± 206.50 (1221.5–1634.5)	$r = + 0.999$ $P = 0.82$
	Female	123	65 (52.84)	1217.4 ± 142.20 (1074.8–1359.2)	
Age	Fawn	24	18 (75.00)	1892.6 ± 182.40 (1709.6–2074.4)	$r = + 0.995$ $P = 0.23$
	Adult	129	64 (49.61)	1341.8 ± 86.80 (1255–1428.6)	

our understanding of the risks of epizootic and zoonotic transmission.

Acknowledgments The authors thankfully acknowledge the financial assistance of the Department of Science and Technology, Govt. of India, New Delhi in conducting this study under the research project. Authors are thankful to Prof. Azra N. Kamili, Centre of Research for Development, University of Kashmir, Srinagar for providing technical support. We thank Department of Wildlife protection Government of Jammu and Kashmir providing the opportunity for conducting research in Dachigam National Park.

References

- Anderson RM, May RM (1979) Population biology of infectious diseases. *Nature* 280:361–367
- Dar GH, Bhagat RC and Khan MA. 2002. Biodiversity of the Kashmir Himalaya. (Valley Book House, Srinagar—190006, J&K), pp. 19
- Dhar DN, Sharma RL, Bansal GC (1982) Gastrointestinal nematodes in sheep in Kashmir. *Vet Parasitol* 11:271–277
- [FAO] Food and Agriculture Organization (1999) State of the world's forests. Food and Agriculture Organization of the United Nations, Italy, p 22
- Gibbs HC (1986) Hypobiosis in parasitic nematodes—an update. *Adv Parasitol* 25:129–174
- Gulland FMD, Fox M (1992) Epidemiology of nematode infections of Soay sheep (*Ovis aries* L.) on St. Kilda. *Parasitology* 105:481–492
- Halvorsen O (1986) Epidemiology of reindeer parasites. *Parasitol Today* 2:334–339
- Khajuria JK, Kapoor PR (2003) Prevalence of parasites in sheep and goats at Kathua-Jammu. *Vet Parasitol* 17:121–126
- Lateef M, Iqbal Z, Jabbar A, Khan MN, Akhtar MS (2005) Epidemiology of trichostrongylid nematode infections in sheep under traditional husbandry system in Pakistan. *Int J Agric Biol* 7:596–600
- Lone BA, Chishti MZ, Ahmad F (2011) Prevalence of coccidia and gastrointestinal nematode infections in goats of Barramulla district of Kashmir valley. *Glob Vet* 7:27–30
- Lone BA, Chishti MZ, Ahmad F, Tak H (2012) A survey of gastrointestinal helminth parasites of slaughtered sheep and goats in ganderbal, Kashmir. *Glob Vet* 8:338–341
- Magona JW, Musisi G (2002) Influence of age, grazing system, season and agroclimatic zone on the prevalence and intensity of gastrointestinal strongylosis in Ugandan goats. *Small Rumin Res* 44:187–192
- [MAFF] Ministry of Agriculture Fisheries and Food (1979) Manual of veterinary parasitology laboratory techniques. Her Majesty's Stationary Office, London, U.K., p 72
- Nashiruddullah N, Darzi MM, Kamil SA, Mir MS, Shahardar RA (2005) Recovery of *Dictyocaulus* species from the lungs of a Kashmir red deer (*Cervus elaphus hanglu*). *Vet Rec* 157:591–592
- Nashiruddullah N, Darzi MM, Shahardar RA, Kamil SA, Mir MS, Mir M (2007) Pathology of spontaneous *Dictyocaulus* sp. infection in hangul (*Cervus elaphus hanglu*), sheep and goat. *J Vet Parasitol* 21:37–40
- Ogunsuri RA, Eysker M (1979) Inhibited development of trichostrongylids of sheep in Northern Nigeria. *Res Vet Sci* 26:108–110
- Pandit BA, Shadardar RA, Darzi MM, Banday MAA, Bhat AS (2003) Survey of gastrointestinal nematodes in sheep of Kashmir Valley. *Indian J Small Rumin* 9:39–42
- Patz JA, Graczyk TK, Geller N, Vittor AY (2000) Effects of environmental change on emerging parasitic diseases. *Int J Parasitol* 30:1395–1405
- Shahadat MA, Karim MJ, Alam MZ, Majumder S (2003) Seasonal distribution of *Haemonchus contortus* in Bengal goats. *Bangladesh Vet* 20:72–76
- Sloss MW, Kemp RL, Zajac AM (1994) Veterinary clinical parasitology, 6th edn. Iowa State University Press, Ames, Iowa, p 198
- Soulsby EJJ. 1982. Helminths, Arthropods and Protozoa of Domestic Animals. 7th Edn. Balliere Tindall, London, 1st Annes Road, East bourne, East Sussex BN21
- Tariq KA, Chishti MZ, Ahmad F, Shawl AS (2008a) The epidemiology of paramphistomosis of sheep (*Ovis aries* L.) in the north west temperate Himalayan region of India. *Vet Res Commun* 32(5):383–391
- Tariq KA, Chishti MZ, Ahmad F, Shawl AS (2008b) Epidemiological study on *Paramphistomum* infection in Goats-Kashmir valley. *World J Agric Sci* 4:61–66
- Tariq KA, Chishti MZ, Ahmad F, Shawl AS (2008c) Epidemiology of gastrointestinal nematodes of sheep managed under traditional husbandry system in Kashmir valley. *Vet Parasitol* 158:138–143
- Tavassoli M, Khoshvaghti H (2010) Helminthes and coccidia infection of wild sheep (*Ovis Ammon Orientalis*) in Kabodan Island of National Park of Urmia Lake, Iran. *Vet Res Forum* 1:26–29
- Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW (1996) Veterinary Parasitology, 2nd edn. Black well Science limited, London, p 307
- Vanimisetti HB, Andrew SL, Zajac AM, Notter DR (2004) Inheritance of fecal egg count and packed cell volume and their relationship with production traits in sheep infected with *Haemonchus contortus*. *J Anim Sci* 82:1602–1611
- Vlasoff A, Leathwick DM, Heath ACG (2001) The epidemiology of nematode infections of sheep. *N Z Vet J* 49:213–221