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Parasitic prevalence in Himalayan black bear (*Ursus thibetanus*) in Kashmir Himalayas

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Abstract
Parasites are considered as significant health problems. During current study, a total of 112 scat samples from Kashmir Himalayas were examined for the presence of parasites from Junly 2014 to June 2015. There was a prevalence of 77.67%. We found eggs of *Baylisascaris transfuga, Ancylostoma* spp. and cyst of *Eimeria* spp. Seasonal data showed significant variation ($p< 0.05$) highest prevalence in Summer. Lowest in spring and Autumn.

Keywords: Black bear, Ursus thibetanus, Baylisascaris transfuga, ova, cyst, scat, seasonal variation.

Introduction
Biodiversity is under heavy pressure globally due to human population growth and an ever-increasing use of natural resources [27, 14]. The species extinction rate is on the increase and mammals are the most vulnerable [16]. Black bear (*Ursus thibetanus*) is among such vulnerable mammals. In India, black bear is found in Jammu and Kashmir (except Ladakh), Himachal Pradesh, Uttar Pradesh, Sikkim, Arunachal Pradesh and other north-eastern states and in the hills of West Bengal [24].
For any successful conservation programme, health care remains corner stone. There is a growing recognition of parasites as a major factor in the biology and conservation of specie [1, 28, 25]. Parasite can lead to mortality or poor health condition. Parasite infection and load in free ranging animals is traditionally done via necropsy or coproscopy [15]. There is no report on the parasitism of black bear in Kashmir. To have insight of parasitism in black bear we undertook this endeavor. This study would also form a foundation for surveillance programs of black bears in Himalayas.

Materials and Methods
This study took place in Kashmir Himalayas an important part of the western Himalayan ecosystem of India. The climate of Kashmir Himalaya is temperate receiving an average precipitation of 65cm, with four distinct seasons a year, spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). The dominant flora is *Prunus avium*, *Prunus persica*, *Salix alba*, *Morus alba*, *Parrotiopsis jacquemontiana*, *Populus alba*, *Pinus wallichiana*, *Quercus robur* etc. Dominant fauna present is *Moschus chrysogaster*, *Ursus thibetanus*, *Panthera pardus*, *Martes flavigula*, *Lutra lutra*, *Marmota caudate*, *Felis chaus*, *Mustela sibirica* etc. [10]. Collection was done from different mountain ranges viz. Zabarwan, Shuhama, Ajas and Banihal. The study was conducted from July 2014 to June 2015.
We analyzed a total of 112 scats during the study. Scats were collected opportunistically, intensively searched for scats in areas where we located bears or found bear sign. We collected fresh bear scats identified by size, shape and color or the presence of nearby bear tracks. Freshness was determined by the moisture and sharpness of features. Scats were handpicked, wearing a rubber glove. Each scat was stored in a plastic bag, labeled with date. Scats were preserved in 5% formalin, to inhibit egg hatching or development of larvae.
Parasite eggs/cyst were recovered from scats by Direct smear method, Concentration by Sedimentation (Charles and Josephine, 1970) [3] and Concentration by Floatation (Charles and Josephine, 1970 and Soulsby, 1982) [3, 26]. Eggs/cyst were observed under

Microscope scanned on 100x and 400x magnification. Eggs were measured by ocular micrometer calibrated with stage micrometer. Egss/cyst were identifie following reference keys (Soulsby, 1982; Kazakoss *et al.*, 1983; Samuel *et al.*, 2001 and Asakawa *et al.*, 2006)

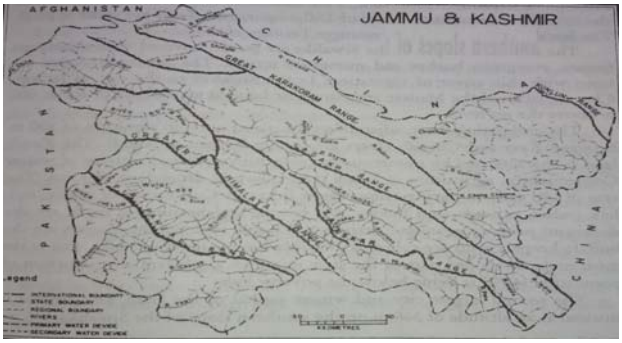

Fig 1: Map of Jammu and Kashmir [10]

Data Analysis

Statistical analysis was done to know the frequency of parasite eggs and cyst present in scat. The percent frequency was calculated by using the formula, FOi (%) = (ni/N)x 100, where N was the total number of scats and ni the number of positive scats. The data was evaluated by ANOVA to know the significance of variance; p-value less than 0.05 were considered significant.

Results

We recovered 3 parasite eggs/cyst from the 87 (77.67%) samples. Two nematode eggs viz. *Baylisascaris transfuga* (fig. 3 and 4) and *Ancylostoma* spp. (fig 5) and a protozoal cyst viz. *Eimeria* spp. (fig 2) were recovered. All parasites identified to the genus or species level have been previously reported in Black bears. *Baylisascaris transfuga* egg has oval shape, brown color, thick shell with irregular albuminous coat and measuring 70.4 x 52.4 µm. *Eimeria* spp. has oval shape, prominent cyst wall, four sporocyst at cyst stage and 21.4 x 19.1 µm size. *Ancylostoma* spp. has elongated egg, 8-16 celled morulla, prominent egg shell and 64.2 x 48.8 µm size. Nematodes and protozoan infection were 77 (68.75%) and 69 (61.60%) samples respectively. The prevalence of different parasitic eggs/cyst of black bear were 69 (61.60%) for *Eimeria* spp., 53 (47.32%) for *Baylisascaris transfuga* and 42 (37.50%) for *Ancylostoma* spp. (Table 1)

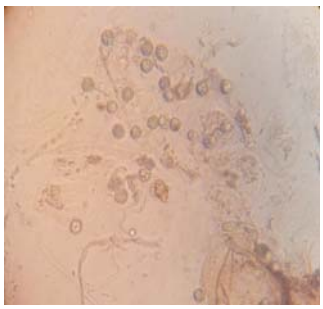

Fig 2: (100x)

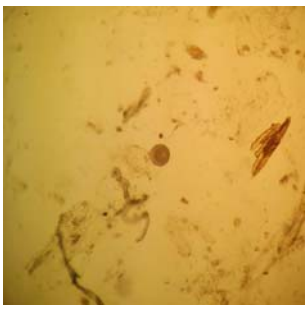

Fig 2: (100x)

Fig 3: (400x)

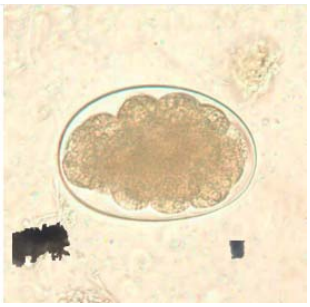

Fig 4: (400x)

Table 1

S. no.	Egg/Cyst	Positive n=112	Prevalence
1	*Eimeria* spp.	69	61.60%
2	*Baylisascaris transfuga*	53	47.32%
3	*Ancylostoma* spp.	42	37.50%
	Total	87	77.67%

Mixed infection was observed in 59 samples. All the three parasitic eggs/cyst were present in 18 (16.07%) samples. *Baylisascaris transfuga* and *Ancylostoam* spp. eggs were found in 5 (4.46%) of samples, *Baylisascaris transfuga* and *Eimeria* spp. egg/cyst were found in 17 (15.17%) of samples and *Eimeria* spp. and *Ancylostoma* spp. egg/cyst were found in 19 (16.96%) of samples.(Table 2)

Table 2

S. no.	Mixed infection	Total scats n=112	Prevalence
1	*Baylisascaris transfuga* and *Eimerai* spp.	17	15.17%
2	*Eimeria* spp. and *Ancylostoma* spp.	19	16.96%
3	*Baylisascaris transfuga* and *Ancylostoma* spp.	05	4.46%
4	*Baylisascaris transfuga*, *Eimeria* and *Ancylostoma* spp.	18	16.07%
	Total mixed infection	59	52.67%

Seasonal prevalence of different parasitic eggs/cysts was analyzed from 51, 39 and 22 samples of summer, atutumn and spring seasons respectively. *Eimeria* spp. was present in 37 (72.54%) samples of summer, 22 (56.41%) samples of autumn and 10 (45.45%) samples of spring. *Baylisascaris transfuga* eggs were found in 31 (60.78%) samples of summer, 16 (41.02%) samples of autumn and 6 (27.27%) samples of spring. *Ancylostoma* spp. was found in 27(52.94%) samples of summer, 9 (23.07%) samples of autumn and 6 (27.27%) samples of spring. (Table 3). Seasonal variation showed significant variation. (p <0.05). Monthly prevalence is shown is Table 4

Table 3

Seasson	Scats examined	Positive			Frequency of occurrence		
		Eimeria spp.	*Baylisascaris transfuga*	*Ancylostoma* spp.	*Eimeria* spp.	*Baylisascaris transfuga*	*Ancylostoma* spp.
Summer	51	37	31	27	72.54	60.78	52.94
Autumn	39	22	16	09	56.41	41.02	23.07
Spring	22	10	06	06	45.45	27.27	27.27
Total	112	69	53	42	61.60	47.32	37.50

Table 4

S.no	Specie	Jan n=0	Feb n=0	Mar n=4	Apr n=9	May n=9	Jun n=12	Jul n=18	Aug n=21	Sep n=17	Oct n=14	Nov n=8	Dec n=0
1	*Eimeria* spp.	-	-	-	+(4)	+(6)	+(7)	+(14)	+(16)	+(11)	+(11)	-	-
2	*Baylisascaris transfuga*	-	-	-	+(2)	+(4)	+(7)	+(10)	+(14)	+(10)	+(6)	-	-
3	*Ancylostoma* spp.	-	-	-	+(3)	+(3)	+(6)	+(10)	+(11)	+(6)	+(3)	-	-

Discussion

Black bears from Kashmir Himalayas have never been studied for parasites in the past. However there are studies on the parasitism of bears outside Kashmir which showed *Eimeria* infections by Hair and Mahrt, 1970 [9], *Baylisascaris transfuga* by Frechette and Rau, 1978 [7] and *Ancylostoma* by Asakawa *et al.,* 2006 [2].

During the present endeavor parasitic infestation was high in summer and low in spring and autumn, it is supported by studies of Frechette and Rau, 1978 [7]. Seasonal variation of *Baylisascaris transfuga* ova is in accordance with the findings of Finnegan, 2009 [5] who found high prevalence during summer compared to spring and autumn. Gau *et al.,* 1999 [8] reported gastrointestinal parasites (*Diphylobthrium* spp., coccidia, Strongyles, and *Baylisascaris* spp.) with 31% prevalence in spring and 58% prevalence in summer, this increase in frequency occurrence from spring to autumn also supports our findings.

Rogers, 1975 and 1976 [20, 21] found bears shed adult parasites before denning. We found zero parasitic infestation during November and March which also supports sheding of parasites before denning. Rush, 1932 [22] and Rausch, 1954 and 1961 [18, 19] speculated that loss and reinfestation of parasites was facilitated through changes in dietary items. Choquette *et al.,* 1969 [4] also was skeptical of changes in dietary items explaining the elimination of parasites before bear hibernation. Frechette and Rau, 1978 [7] considered a cessation of feeding to explain the changes they observed in the prevalence of bear parasites.

The eggs of parasite eggs are resistant to freezing and those shed in the fall may act as a source of infection the following spring when bears come out denning (Kutz, 2004) [14]. Larvae that overwinter in the intestinal mucosa of bears and mature upon emergence from hibernation shedding eggs into the environment may also serve as a source of new infection (Frechette and Rau, 1978) [7]

Despite the information acquired, the impact of parasites of black bear health is poorly understood. High intensity infection can be dramatic to bears in crowded conditions. Examinations of bear scats provide useful data on the risk of environmental occurrence of parasites which may have zoonotic potential. Our results suggest contamination is high, though in wild habitats. It must be kept in mind humans are at a risk of serious infection following accidental ingestion of eggs. It's mandatory that personal hygiene is strictly observed.

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