

# Results of a preliminary examination of parasites in the feces of Tibetan antelopes (*Pantholops hodgsoni*), blue sheep (*Pseudois nayaur*) and domestic sheep (*Ovis aries*) in the Tibetan Plateau

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## Introduction

With an area of 2.5 million square kilometers at an average elevation of over 4,500 m, the Tibetan Plateau is the biggest and highest plateau in the world. Covering most of Tibet and Qinghai provinces in China and Ladakh in India, it is a unique ecosystem composed of arid grasslands interspersed with mountain ranges and large brackish lakes. The northwestern part upholds an exceptional community of large wild mammals, including six endangered ungulate species: the Tibetan antelope also known as chiru (*Pantholops hodgsoni*), the Tibetan wild ass or kiang (*Equus kiang*), the Tibetan gazelle (*Procapra picticaudata*), the wild yak (*Bos mutus*), the blue sheep (*Pseudois nayaur*) and the Tibetan argali (*Ovis ammon hodgsoni*). Although living in a very remote area these species face many threats, which endanger them and the rangeland ecosystem that supports them.

Until the 1980's, the wildlife and ecosystems of the Tibetan Plateau had been little studied. Then surveys led by G. B. Schaller and his Chinese coworkers have provided information on rangelands, wildlife, and pastoral systems in the area. However, the health status of both wild and domestic ungulates in this part of the world and — of even greater concern to biologists — the risk of disease transmission between them, is still poorly known.

To enhance our understanding of the health factor in the ecology of Tibetan wildlife, opportunities were taken during recent field trips to collect fresh fecal droppings of Tibetan antelopes and blue sheep in order to evaluate quantitatively the level of shedding of helminth eggs and unsporulated coccidian oocysts. This investigation was not triggered by a clinical observation of likely high level of parasite infestation (such as visible signs of diarrhea, chronic emaciation or even abnormal mortality) in these

species, but as an exercise to learn a little more about the community of parasites hosted by Tibetan wildlife following the very preliminary results provided by Schaller (1998) who reported parasite findings in 17 fecal samples from 8 different species of ungulates in the Tibetan Plateau.

The present report intends to provide preliminary results of a parasitology study in Tibetan wildlife, but more importantly to offer thinking material to biologists and veterinarians interested at studying how and to which extent parasites may affect the ecology of wild herbivores in the Tibetan Plateau.

## Materials and methods

Fecal samples were collected from the ground in areas recently utilized by Tibetan antelope or blue sheep. Only droppings that were soft, warm, not frozen in winter and moist and glistening in summer were selected. Adhering soil was brushed away from fecal pellets and 4-5 g were placed in 15-ml plastic containers with about 5 ml of formalin 10% (prepared as 1 volume of formaldehyde 37-40% in 9 volumes of bottled mineral water). Examination of these samples for parasite eggs was done at the Institute of Zoology of the Chinese Academy of Science in Beijing. Samples were allowed to sediment for two days before being processed. Supernatant formalin was then carefully removed from vials to avoid re-suspending the feces. Formalin-saturated feces were thoroughly homogenized,  $1.0\text{g}\pm 0.09\text{ g}$ , passed through a 500  $\mu\text{m}$ -mesh strainer and mixed with 14 ml of a flotation solution (360 g of saccharose and 540 g of sodium nitrate in 1000 ml of water) at 20°C (Di Felice and Ferretti, 1962). Density of the solution was checked once a day with a glass hydrometer (range 1.300–1.400) and maintained at 1.320 ( $\pm 0.02$ ) throughout the work. Immediately after mixing, 0.30 ml of the suspension was introduced in the two cells of a McMaster counting slide (Hawksley, UK). Flotation process was allowed to operate for 5 minutes before the counting cell was examined under the  $\times 10$  objective of a light microscope (Leica DMLS, Germany).

All eggs which lay within the lined centimeter square of the counting chambers were counted. Each counted egg represented '50 eggs per gram of feces'. This calculation was based on the fact that the depth of chamber is 1.5 mm and consequently the volume of fluid examined is 0.15 ml, which is 1/100<sup>th</sup> of the original volume of 15 ml, made up of 14 ml of flotation solution and 1 g of feces. Therefore each egg counted represented 100 eggs per 1 g of feces. Because two chambers were systematically counted the total count was multiplied by 50 instead of 100. We repeated counts two times for each fecal sample (i.e.  $2 \times 1\text{ g}$  of feces examined for each sampled animal) and averaged the results. The main drawback of this method is its lack of sensitivity, since infestation rates lower than 50 eggs per gram cannot be detected.

# Results

The study included 77 samples of which 21 were obtained from domestic sheep, four from domestic yak, 32 from Tibetan antelope and 20 from blue sheep (Table 1). Tibetan antelopes were sampled between 15 June and 3 July 2009 in Tibet and blue sheep between 20 October and 6 November 2009 in Qinghai.

We found unsporulated coccidian oocysts (possibly *Eimeria*) and eggs of gastrointestinal nematodes in Tibetan antelopes, blue sheep and domestic sheep. Nematode eggs belonged to Trichostrongylidae, Chabertiidae, Oxyuridae and Trichuridae families, including probably *Ostertagia* sp., *Trichostrongylus* sp., *Oesophagostomum* sp., *Enterobius* sp., *Trichuris* sp. and *Capillaria* sp. genus. Yet, because we are not familiar with the morphology of nematode eggs in species from the Tibetan Plateau, we decided not to discriminate the number of nematode eggs per gram (epg) of feces according to nematode families. Compared to the preliminary results of parasite investigations in feces of four Tibetan antelopes (Schaller 1998), we have confirmed the presence of *Enterobius* sp. ( $N=1$ ) and coccidia parasites ( $N=28$ ) and have also added Strongyle ( $N=18$ ), *Trichuris* sp. ( $N=5$ ) and possibly the tapeworm *Moniezia* sp. ( $N=2$ ) to the list of parasites infesting this species. In general the parasite community observed in Tibetan antelopes resembles what has been observed in other wild mountain ungulates in Central Asia, such as Marco Polo Sheep (*Ovis ammon polii*) in Pamirs (Ostrowski et al. 2009). The four sampled domestic yak had neither nematode eggs nor coccidian oocysts in their feces.

The prevalence of unsporulated coccidian oocysts was very high in all tested species except in the domestic yak. We found infestation rates of 95.2% (95%CI: 76.2-99.9%), 84.4% (95%CI: 67.2-94.7%), and 73.6% (95%CI: 48.8-90.8%) in domestic sheep, Tibetan antelope and blue sheep, respectively (Table 2). The median number of oocysts per gram (opg) was 650 (min-max: <50 – 11,800 opg) in domestic sheep, 200 (min-max: <50 – 4,050 opg) in Tibetan antelope, and 50 (min-max: <50 – 250 opg) in blue sheep.

Prevalence of gastrointestinal nematode eggs was the highest compared to other examined herbivores in domestic sheep, with 71.4% (95%CI: 47.8-88.7%) of these animals infested. It was also relatively high in Tibetan antelopes, with 40.6% (95%CI: 23.7-59.3%) of the animals shedding eggs, compared to only one blue sheep (5.2%; 95%CI: 0.1-26%). However, interspecific comparison is not very meaningful since Tibetan antelopes and blue sheep were sampled in two very distant areas, in different habitats and at two different times of the year. The median number of epg was 100 (min-max: <50 - 900 epg) in domestic sheep and 50 (min-max: <50 - 600 epg) in Tibetan antelopes.

Intraspecific comparison of opg and epg according to collection sites provided interesting results. There was no significant difference in coccidian infestation rate between blue sheep sampled near Baiyu, near Gongya monastery or in Gongsu gumpa ( $P=0.39$ , Kruskal-Wallis One Way AOV). However there was a significant difference in opg in Tibetan antelopes between sampled populations ( $P=0.02$ ).

Table 1. Origin, date of collection, and number of fecal samples of wild and domestic ungulates collected during the present study, Tibetan Plateau, China.

Locality	Location	Date of collection	Domestic sheep and goat	Domestic yak	Tibetan antelope	Blue sheep	Total
Rongma	34°24'N-86°38'E	15, 22, 23 June 09	6	0	18	0	24
Xainza	30°55'N-89°21'E	2 July 09	7	0	7	0	14
Nyima	31°35'N-87°28'E	30 June 09	6	0	7	0	13
Near Baiyu <sup>1</sup>	33°14'N-101°01'E	20 October 09	0	0	0	5	5
Gongya monastery <sup>2</sup>	31°58'N-96°36'E	28 October 09	0	2	0	8	10
Gongsa gumpa <sup>3</sup>	34°09'N-94°12'E	1-6 November 09	2	2	0	7	11
Total			21	4	32	20	77

<sup>1</sup>No domestic sheep in the area since 1990's, yak present during summer.

<sup>2</sup>Domestic sheep phased out in past 5 years, yak present.

Table 2. Median number of nematode eggs and coccidian oocysts in feces of Tibetan antelope, blue sheep and domestic sheep from various localities in Tibet and Qinghai.

Locality Location Date of collection	Species	Median number of gastrointestinal nematode eggs per gram of feces	Median number of coccidian oocysts per gram of feces
Rongma 34°24'N-86°38'E 22-23 June 09	Tibetan antelope	0	150
	Domestic sheep	0	150
Xainza 30°55'N-89°21'E 2 July 09	Tibetan antelope	250	200
	Domestic sheep	450	650
Nyima 31°35'N-87°28'E 30 June 09	Tibetan antelope	150	1150
	Domestic sheep	125	2725
Near Baiyu 33°14'N-101°01'E 20 October 09	Blue sheep	0	100
Gongya monastery 31°58'N-96°36'E 28 October 09	Blue sheep	0	100
Gongsa gumpa 34°09'N-94°12'E 1-6 November 09	Blue sheep	0	50
	Domestic sheep	0	50

Migratory chirus in Rongma had lower coccidia infestation rates compared to resident animals in Nyima but not compared to those sampled in Xainza ( $P=0.05$ ; K-W pairwise comparison test). Interestingly a similar trend was observed between domestic sheep populations, with a significantly lower coccidia infestation rate of domestic sheep in Rongma compared to those in Nyima ( $P<0.001$ , Kruskal-Wallis One Way ANOVA).

Intraspecific comparison of egg shed in feces varied between populations of Tibetan antelopes; migratory animals sampled in Rongma shedding significantly less eggs than those in Xainza ( $P<0.01$ ), but not significantly less than animals from Nyima. We observed a similar trend in egg in domestic sheep sampled in these three localities.

## Discussion

The laboratory method we have used in this study is fairly simple to put in place, and provides coarse but reliable results. Although it lacks detection sensitivity, since infestation rates lower than 50 epg (or opg) cannot be detected, the method is robust, precise and easy to develop with minimal laboratory settings, including in the field. It is however important to respect carefully the different steps of the procedure. For example, if the examination of feces is to take place several months after collection time, it is essential to use a buffered formalin solution as preservative for feces. In the present case, because the formalin aqueous solutions used were not buffered, nematode eggs had unusual shapes or even broken membranes and shells, possibly because of the effect of formic acid, a derivate from formaldehyde that builds up quickly if the solution is not buffered. Such morphological deteriorations may render egg detection and identification very difficult with a corollary risk of underestimating infestation levels. Easy methods to buffer (at pH=7) one liter of distilled water consist at using specific ready-prepared buffer salts (available commercially) or to add 4.5 grams of sodium phosphate (monobasic) and 3.6 grams of sodium hydroxide, or as a last resort to add 4 grams of sodium chloride (table salt).

Several improvements could be brought to this type of study to better understand the community of parasites hosted by Tibetan wildlife. One would consist at learning accurately the morphological appearance of the eggs of the different genus of nematode parasites occurring in Tibetan wildlife. Because our knowledge is largely based on extrapolating what is known of the morphology of these eggs in other parts of Eurasia we have not discriminated nematode eggs according to nematode genus in the present study. Being able to accurately discriminate these genus could however prove very interesting to better understand the occurrence of different nematode groups according to species, season, breeding status and a variety of other factors. For those interested in pursuing such research, the initial step would be to compare egg morphology in feces and adult parasites in the digestive tract collected concomitantly on freshly dead individuals. Another possibility, more complicated, would

consist at 'growing' parasites in laboratory settings to get familiar with the morphology of their different development stages (eggs, larvae, and adults). Also another improvement to coproscopical studies of parasites in Tibetan ungulates would consist at using a specific method to isolate and count lungworm larvae in addition to gastrointestinal nematode eggs (for example the Baermann-Wetzel method).

We have made interesting preliminary observations on the interspecific and intraspecific variations of parasite eggs and coccidian oocysts shed in feces of wild ungulates in the Tibetan Plateau. Overall domestic sheep had higher egg/oocyst counts than Tibetan antelopes which in turn had higher counts than blue sheep. Yet because samples were taken at different seasons our data set does not allow conducting more in-depth analyses of possible variations between Tibetan antelopes and blue sheep. Intraspecific comparisons between populations of Tibetan antelopes showed that nematode eggs and coccidia shedding were significantly lower in migrating animals than in one of the two resident populations tested. The fact that this difference was not significant with both resident populations and differing also for nematode eggs and coccidian oocysts, is most likely a consequence of the small sample size for resident populations ( $N=7$ ). A very similar trend was observed between groups of domestic sheep sampled, suggesting (but not proving) that the level of intestinal parasites in the Tibetan antelope could be correlated with livestock infestation rates. However, far more in-depth studies are necessary to demonstrate this relationship. One initial step would be to collect in resident and migratory populations of chirus, as well as in cohabiting livestock, adult specimens of nematodes from freshly dead animals in order to confirm that they are, at least locally, the same species across wild and domestic hosts. A similar study could also be done for coccidia from fecal material with the help of molecular methods. The next step would then require collecting fecal samples on large sample sizes, throughout seasons and from different age classes and both sexes. Such extensive work could very well constitute the frame of a student research effort, the principal goal being to elucidate the relationship between parasite communities and the ecology of Tibetan antelopes.

Similar studies of parasites could be carried out on almost every wild ungulate species in the Tibetan Plateau. In particular, it would be very useful and relevant to their conservation to investigate the effect of parasites on species exposed to a high level of fragmentation and competition with livestock. The endangered Przewalski's gazelles (*Procapra przewalskii*) scattered in several populations over a relatively small area around Qinghai Lake would certainly qualify for a more in-depth study of its parasite community.

Density-dependent processes usually govern transmission of digestive tract parasites. As a consequence parasites that affect host communities with low densities, such as in ungulate species of the Tibetan Plateau, have evolved relatively low virulence but high transmission rates. In other words they are relatively benign but have retained high fecundity and longevity in their different infectious stages (ovum/egg, larval adult). In such circumstances one may legitimately question whether these parasites are of any health concern to wild ungulates in the Tibetan Plateau. There is however a growing number of peer-reviewed scientific literatures showing that macro-parasite and other infectious agents

can greatly influence the population dynamics of their hosts. This situation often occurs when hosts are restricted in range usually in conjunctions with human modifications of the environment. We believe this threat is particularly relevant in the case of parasites adapted to low-density host populations, such as in the Tibetan Plateau where range fragmentation progresses rapidly. Under such circumstances the adaptive high longevity and fecundity of native parasites may translate into rapidly and 'abnormally' increasing burden and health concerns at population level. Because health problems may affect host abundance, the effect of macro-parasite infestation on threatened species<sup>0</sup> is directly relevant to conservation biologists. There is indeed an urgent need to measure the effects of parasites and other infectious agents in threatened wildlife communities of the Tibetan Plateau. These investigations are needed to better adjust conservation strategies and management practices for this unique biodiversity.

We hope that this preliminary work will trigger further interests within the talented community of Chinese scientists.

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