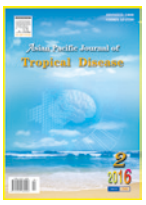
HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Epidemiological investigation doi: 10.1016/S2222-1808(15)61001-5

Prevalence of gastrointestinal and liver parasites in yaks in the cold desert area of lower Mustang, Nepal

Krishna Prasad Acharya[1,2*], Bimal Kumar Nirmal[3], Krishna Kaphle[4], Manoj Kumar Mahato[1,2], Ganga Prasad Yadav[2], Hari Bahadur Rana[5]

[1]*Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal*

[2]*Tribhuwan University-Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal*

[3]*Directorate of Livestock Production, Government of Nepal, Hariharbhawan, Lalitpur, Nepal*

[4]*Department of Theriogenology, Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal*

[5]*Department of Veterinary Microbiology and Parasitology, Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal*

ARTICLE INFO

Article history:
Received 3 Dec 2015
Received in revised form 15 Dec 2015
Accepted 28 Dec 2015
Available online 21 Jan 2016

Keywords:
Gastrointestinal parasites
Liver parasites
Yak
Desert
Mustang
Nepal

ABSTRACT

Objective: To determine the prevalence and associated risk factors of gastrointestinal and liver parasites in yak in the cold desert area of the Mustang District, Nepal.

Methods: Fecal samples were collected over a period of three months from 96 yaks from the high Himalayan District of Mustang, Nepal. The samples were tested for the presence of parasites by direct smear, sedimentation, and floatation techniques. Yak herders were surveyed with pre-tested questionnaires by participatory appraisals to explore their knowledge and awareness of parasitic diseases and health management.

Results: Examination of fecal samples revealed that 82 were positive for one or more parasites, giving an overall prevalence of 85.42%, in which 6.25% had single and 79.17% had multiple parasitic infection. Animals with poor body condition scores and young age were more susceptible than their counterparts. Inferior body condition scores were attributed to parasitic burden. No significant difference was noted between worm burden and either the sex of the animal or the altitude.

Conclusions: A high proportion of yaks in the lower Mustang Region of Nepal suffered from the mild to moderate parasitic infection, significant enough to contaminate the pasture and spread infection to healthy animals. Complementary studies are needed to establish the impact of parasitism on productive performance. Furthermore, nutrition and health management, including regular and strategic parasite monitoring programs are needed for better health and productivity.

1. Introduction

Among 17 species of domesticated animals in Nepal, yak holds prime position in alpine context. They are a major component of Himalayan livestock farming and an important source of income for pastoral tribes living in the Nepalese highlands. Moreover, yak and yak hybrids are major sources of milk and meat and are integral to the cultural and social activities of herding societies[1,2]. There are 70 165 yaks in Nepal[3], with 4 145 in the Mustang District, including 900–1 000 yaks in lower Mustang[4]. Although yak and chauri are credited for their high tolerance or resistance to disease and the environment, parasitic infection is a major cause of production loss, mainly due to severe weight loss, poor milk and meat production, and impaired reproductive performance. Helminths are the most prevalent when the animals are in temperate and subalpine pastures between October and May. This period is followed by travel to higher land and mating, typically between June and October. However, it is unknown how travel, reproduction, diverted nutrient needs, and herd behavior dynamism are related to the parasitism cycle, but the problem is especially common in calves where parasites cause heavy mortality[5]. Helminths cause direct loss due to acute illness and indirect losses due to poor growth, poor reproductive efficiency, loss of production, and poor feed conversion (chronic cases).

*Corresponding author: Krishna Prasad Acharya, Post Graduate Student, Department of Animal Breeding and Biotechnology, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal.
Tel: +977-9845721775
E-mail: kriaasedu@gmail.com
The journal implements double-blind peer review practiced by specially invited international editorial board members.

Most yaks are not involved in treatment program because worm infection does not cause any specific symptoms that might alert yak herders to the problem[6]. Information on the prevalence of gastrointestinal helminth infections is limited. Some studies have revealed an incidence of helminthic infection in chauri and yak of Nepal[2,7-10]. However, there are no data on helminth parasites in yak in the Mustang District. We therefore aimed to determine the prevalence and associated risk factors of gastrointestinal and liver parasites in the northern mountainous region of Mustang, Nepal. These data are useful for the planning measures to control gastrointestinal parasitic diseases and have a direct impact on productive performance of yak and chauri.

2. Materials and methods

2.1. Sample size estimation

The sample size was determined using Daniel[11]:

$$n = \frac{Z^2_{1-\alpha/2} \times p\,(1-p)}{e^2}$$

Where Z = 1.96, p is the expected prevalence of 5.47%[12], and e is the maximum tolerable error of 0.05. This resulted in a required sample size of 79.45, *i.e.*, 80 animals. However, we had taken 96 yak samples to increase the precision of the study.

2.2. Study area and animal population

This study was conducted in lower Mustang (Lete, Kowang, Tukuche, Marpha Village), Nepal. The study region lies between the 28°37' N, 83°36' E and 28°50' N, 83°58' E in the trans-Himalayan arid zone and is bounded to the east by Manang, the west by Dolpa, the south by Myagdi, and the north by Upper Mustang. Six herds and 50% of the population of each herd were chosen at random for sample collection. Each yak herder completed a pre-tested questionnaire to assess the variables associated with health care and awareness about parasitic diseases by farmers (n = 32).

2.3. Fecal sample collection and examination

During February to March 2014, a total of 96 fecal samples (10% of population of yaks) were collected from yaks in lower Mustang. Samples were collected either directly from the rectum or freshly voided feces early in the morning. Each fecal sample was immediately put into a zip-lock plastic bag, stored in an icebox, transported to the regional veterinary laboratory of Pokhara, and stored in a refrigerator until further processing. Fecal samples were examined by the direct smear, sedimentation, and flotation methods as per standard techniques[13]. Quantitative fecal examination was carried out by a modified McMaster technique[14] with a sensitivity of 50 eggs per gram (EPG)/oocyst per gram. For the floatation technique, saturated zinc sulfate solution (specific gravity 1.15) was used for trematodes and saturated sodium chloride solution (specific gravity 1.2) was used for cestodes and nematodes. Helminth eggs were identified by low and high power microscopy according to the size of the eggs and morphological characteristics[13].

2.4. Statistical analysis

Differences in individual parasitic burden between altitude, body condition score, age, and sex were determined using ANOVA test from contingency tables. Analysis was performed using IBM SPSS version 19.0. The infection prevalence and questionnaires were also analyzed using descriptive statistics.

3. Results

Out of 96 fecal samples, 82 were positive for one or more parasites; thus, the overall prevalence was 85.42%, in which 6.25% were single parasitic infections, and 79.17% were multiple parasitic infections. Ten different types of parasites were found: strongyles (46.87%), *Toxocara* (29.17%), *Haemonchus* (18.75%), *Taenia* (18.75%), *Fasciola* (16.67%), *Moniezia* (15.62%), *Dicrocoelium* (11.46%), *Paramphistomum* (9.37%), *Trichuris* (9.37%), and *Nematodirus* (2.08%) (Table 1).

Table 1
Prevalence of helminthes and protozoan parasites in yak of lower Mustang, Nepal.

Parasites	Examined yaks	Positive yaks [*n* (%)]
Gastrointestinal strongyle	96	45 (46.87)
Toxocara	96	28 (29.17)
Hemonchus	96	18 (18.75)
Trichuris	96	9 (9.37)
Nematodirus	96	2 (2.08)
Fasciola	96	16 (16.67)
Paramphistomum	96	9 (9.37)
Dicrocoelium	96	11 (11.46)
Moniezia	96	15 (15.62)
Taenia	96	18 (18.75)
Eimeria	96	19 (19.79)

Parasite prevalence was higher in females, animals residing at lower altitudes, those with poor body condition, and those either younger or older than their counterparts (Table 2).

Table 2
Percentage prevalence of gastrointestinal and liver parasites in desert area of Lower Mustang, Nepal.

Parameters		Examined samples (*n*)	Infected animals (*n*)	Infection (%)
Age	Below 2 years	24	20	83.33
	2–7 years	65	55	84.61
	Above 7 years	7	7	100.00
	Total	96	82	85.42
Sex	Male	29	23	79.31
	Female	67	59	88.05
	Total	96	82	85.42
Body condition	Thin (< 2.5)	15	11	73.33
	Moderate (2.5 ⩽ 3.0)	81	71	87.65
	Total	96	82	85.42
Altitude	Below 3000 m a.s.l (TP)	37	34	91.89
	3000–4000 m a.s.l (SA)	46	39	84.78
	Above 4000 m a.s.l (AP)	13	9	69.23
	Total	96	82	85.42

a.s.l: Above sea level; TP: Temperate pasture; SA: Subalpine area; AP: Alpine pasture.

Of the isolated parasites, nematodes were most prevalent (78.13%) followed by trematodes (35.42%) and cestodes (34.38%) (Table3).

Of the overall prevalence of 85.42%, 22.92% were recorded in animals with a history of anthelminthic treatment and the remainder (62.5%) were recorded in animals with no history of

Table 3
Genera wise prevalence of different parasites in yak of lower Mustang, Nepal. *n* (%).

Parameters		EPG range	Parasitic infection		Trematodes	Nematodes	Cestodes
			Single parasitic infection	Multiple parasitic infection			
Age	Below 2 years	0–300	1 (4.17)	19 (79.17)	8 (33.33)	18 (75.00)	2 (8.34)
	2–7 years	0–200	5 (7.69)	50 (76.92)	22 (33.85)	50 (76.92)	26 (40.00)
	Above 7 years	100–1 000	0 (0.00)	7 (100.00)	4 (57.14)	7 (100.00)	5 (71.43)
	Total	0–1 000	6 (6.25)	76 (79.17)	34 (35.42)	75 (78.13)	33 (34.38)
Sex	Male	0–400	2 (6.89)	21 (72.41)	12 (41.38)	17 (58.62)	18 (62.07)
	Female	0–1 000	4 (5.97)	55 (58.33)	22 (32.84)	58 (86.57)	15 (22.39)
	Total	0–1 000	6 (6.25)	76 (79.17)	34 (35.42)	75 (78.13)	33 (34.38)
Body condition	Thin (< 2.5)	0–1 000	1 (6.67)	10 (10.41)	8 (53.34)	9 (60.00)	5 (33.34)
	Moderate (2.5 ⩽ 3.0)	0–400	5 (6.17)	66 (68.75)	26 (32.09)	66 (92.96)	28 (34.57)
	Total	0–1 000	6 (6.25)	76 (79.17)	34 (35.42)	75 (78.13)	33 (34.38)
Altitude	Below 3 000 m a.s.l (Temperate pasture)	0–1 000	1 (2.70)	33 (34.37)	22 (59.46)	31 (83.78)	12 (32.43)
	3 000–4 000 m a.s.l (Subalpine pasture)	0–300	3 (6.52)	36 (37.50)	10 (21.74)	38 (82.61)	17 (36.96)
	Above 4 000 m a.s.l (Alpine pasture)	0–300	2 (15.38)	7 (53.85)	2 (15.38)	6 (46.14)	4 (30.77)
	Total	0–1 000	6 (6.25)	76 (79.17)	34 (35.42)	75 (78.13)	33 (34.38)

a.s.l: Above sea level.

treatment (Table 4). The prevalence of helminths in yaks with a history of anthelminthic treatment was lower than that in animals with no such history (Table 4).

Table 4
Deworming history and prevalence of parasites.

Parameters	Number of yaks tested	Treated *n* (%)	Untreated *n* (%)	Total *n* (%)	*Chi*-square	*P*-value
Positive	32	22 (22.92)	60 (62.5)	82 (85.42)	3.41	0.02
Negative	64	10 (10.41)	4 (4.17)	14 (14.58)		
Total	96	32 (33.33)	64 (66.67)	96 (100.00)		

Quantitative examination of fecal samples showed that the majority of yaks (62/96) were infected to a low degree (< 500 EPG), 20 (20.83%) yaks were moderately infected, and none of the yak were heavily infected (> 1 000 EPG). The mean burden of different helminthes was assessed according to age, sex, and body condition. There was a higher mean burden of worms in animals over 7 years of age, females, and in animals with a lower body condition (Table 5). The mean burden of strongyles and *Fasciola* were significantly different according to age, but only *Fasciola* were significantly different according to the body condition of animals. There were no significant differences in the mean EPGs of different helminths between animals of different sex (Table 5).

Table 5
Mean parasitic burden of different helminthes.

Parameters		*Toxocara* EPG	Strongyle EPG	*Fasciola* EPG	*Hemonchus* EPG	*Moniezia* EPG	Total EPG
Age	Below 2 years	133.33 ± 19.39	65.83 ± 16.07*	30.33 ± 6.13*	25.83 ± 7.90	44.68 ± 27.30	300.00 ± 34.05
	2–7 years	72.18 ± 74.48	90.76 ± 7.89*	50.91 ± 22.53*	24.61 ± 16.87	32.30 ± 10.94	270.15 ± 23.81
	Above 7 years	185.00 ± 73.08	214.28 ± 49.97*	228.57 ± 1.11*	57.14 ± 23.38	42.86 ± 7.86	728.87 ± 80.81
Sex	Male	69.86 ± 36.37	86.20 ± 38.90	37.93 ± 27.52	37.05 ± 24.94	44.82 ± 18.31	275.86 ± 42.59
	Female	74.00 ± 12.50	68.40 ± 7.15	75.00 ± 22.50	42.16 ± 14.42	52.38 ± 21.35	311.94 ± 26.86
Body condition	Thin (< 2.5)	52.00 ± 57.73	106.03 ± 60.61	150.00 ± 52.00*	50.00 ± 88.00	35.00 ± 46.29	393.33 ± 90.22
	Moderate (2.5 ⩽ 3.0)	76.67 ± 18.43	64.33 ± 3.89	52.36 ± 18.10*	45.48 ± 8.77	54.55 ± 13.04	293.83 ± 20.81

*: Statistically significant; EPG presented as mean ± SE.

4. Discussion

The overall prevalence of parasites in yak of this region of Nepal was 85.42%, which is in agreement with other reports[9,15,16] around the world. However, this is higher than that reported by Bam *et al.*[12] and Goswami *et al.*[17] in Arunachal Pradesh, India. The mean EPG (310.00 ± 22.82) in yak of the Mustang was higher than that reported by Goswami *et al.*[17] and Rahman *et al.*[18] in India, who reported 181.50 and 160.56 EPG, respectively. The higher infection rate and EPG in yaks in Mustang might be due to the conducive environment, continuous exposure, or availability of infective larvae on the pasture. Conversely, the low rate of infection and lower EPG in animals residing at subalpine and alpine levels might be due to unfavorable climatic conditions inhibiting the propagation of parasite eggs and larvae. Rahman *et al.*[18] observed similar in India.

Gastrointestinal strongyle infection was most common (47%), in agreement with several previous studies[9,16,18,19]. *Fasciola* was found in 17% of animals, especially below 3 000 m, similar to Joshi *et al.*[1], Byanju *et al.*[9] and Weiner *et al.*[20]. Our observation of *Eimeria* in some animals was supported by Goswami *et al.*[17] and in China by Hogg[21]. However, the overall parasitic infection of yak was lower than reported by Shrestha and Bindari[10] in yak-cattle hybrids in the Ramechhap District, which might be due to fecal sample collection during the rainy season, which is more favorable for parasite development. Alternatively, it might be due to the study of chauri (yak-cattle), which usually graze at lower altitudes than yak. The higher prevalence in yak at lower altitudes (< 3 000 m) might also be due to the common environment shared by the local cattles, goats, and yaks resulting in worms being contracted by yak[17,18,22].

Helminths were more common in females than males, but this was not statistically significant. The mean burden of *Fasciola* and strongyles were significantly different according to the age of the animals, while only the mean burden of *Fasciola* was significantly different according to the body condition of the animals. Coprological studies have shown that yak in Mustang harbored some zoonotic helminths (*Fasciola hepatica*, *Toxocara*

vitulorum, *Trichuris*, and Amphistomes), of which *Toxocara* was the commonest (Table1). Although *Fasciola* is not transmitted directly via eggs and eggs in feces are not a source of zoonotic infection, the favorable environment of the winter pastures might be conducive for the propagation of *Fasciola* larvae. Therefore, on the winter pastures, certain larvae might be of zoonotic importance. Owing to the poor sanitation and close contact with animals, nomads are vulnerable to these zoonotic worms. The *Taenia* spp. observed in this study might represent pass-through (*i.e.*, not parasitic) due to direct contact with herd dogs and contamination of grazing pastures.

The absence of deworming in most farms is in agreement with Degen *et al*.[23] and Acharya *et al*.[24]. Although most herders were aware of gastrointestinal parasites, only two performed routine deworming. Herders usually deworm their young animals, but sub-adult and adult animals are dewormed only when they're symptomatic as supported by our previous findings[24,25].

The higher parasitic load may be a major contributing factor towards a poor body condition, which might predispose yak to a number of hazardous infections and poor health and physical condition, resulting in yak decline in Mustang. Our finding indicated that majority of yaks are infected with mild to moderate worm burden. Since, relatively high proportions of yaks are suffering from multiple parasitic infections including parasites of zoonotic importance regular de-worming and nutritional interventions are strongly recommended. Moreover, further research on the distribution of parasites, their diversity, the extent of reproductive and productive wastage due to them and sustainable approach in all aspects including the practice of medicine for control and treatment of yak diseases has to be advocated.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are highly thankful to the Institute of Agriculture and Animal Science (IAAS), the National Livestock Breeding Center (NLBC) Pokhara and District Livestock Service Office Jomsom for their technical help in carrying out this research. Dr. D. K. Singh, chairperson of the Internship Advisory Committee, bachelor of Veterinary Science and Animal Husbandry (B.V. Sc. & A.H), IAAS, Tribhuwan University (TU), is given special thanks for allowing this research during the internship period of the first author. We also thank all the yak owners and shepherds who provided us with valuable information about yak rearing. We are also indebted to Mohanlal Thakali and Dhan Prasad Ghale of Marpha who helped to rescue us from a snow cave in Pancholek of Marpha.

References

[1] Joshi DD, Lund PN, Miller DJ, Shrestha SK. Yak production in Nepal. In: Rongchang Z, Jianlin H, Jianping W, editors. *Proceedings of the first congress on yak held in Lanzhou, P.R. China*. Lanzhou: Supplement of Journal of Gansu Agricultural University; 1994, p. 105-12.

[2] Das DN, Das BC, Sarkar M, Mohanty TK, Mondal DB, Singh BP, et al. Yak and its domestication. *Asian Agric History* 1998; **2**: 143-56.

[3] Ministry of Agricultural Development. Statistical information on Nepalese agriculture. Singha Durbar: Ministry of Agricultural Development; 2012. [Online] Available from: http://www.moad.gov.np/downloadfile/yearbook2012_1363677455.pdf [Accessed on 20th November, 2015]

[4] District Livestock Service Office. *The annual report*. Jomsom: District Livestock Service Office; 2013, p. 6-7.

[5] Kharel M. Yak, jholang, mountain cattle and their crossbreds in Nepal. Proceedings of Fourth Global Conference on Conservation of Domestic Animal Genetic Resources; 1998 Aug 17-21; NARC, Nepal and RBI, UK.

[6] Dorji T, Tshering G. Ethnoveterinary practices in Bumthang Dzongkhag. *J Renew Nat Resour Bhutan* 1999; **1**(1): 104-15.

[7] Alirol P. Animal husbandry in the Ganesh Himal Region: an essay in ecological synthesis. *J Inst Nepal Asian Stud* 1976; **3**: 47-61.

[8] Joshi DD. *Yak and Chauri husbandry in Nepal*. Kathmandu: H.M. Government Press; 1982.

[9] Byanju R, Shrestha SP, Khanal DR. Prevalence of gastrointestinal parasites in yaks of Lehe VDC, Manaslu Conservation Area. *Nepal J Sci Technol* 2011; **12**: 366-9.

[10] Shrestha S, Bindari YR. Prevalence of gastrointestinal parasites in chauries of Gumdel VDC of Ramechhap District, Nepal. *Net J Agric Sci* 2013; **1**: 73-4.

[11] Daniel WW. *Biostatistics: a foundation for analysis in the health sciences*. 7th ed. NewYork: Wiley; 1999.

[12] Bam J, Deori S, Paul V, Bhattacharya D, Bera AK, Bora L, et al. Seasonal prevalence of parasitic infectionof yaks in Arunanchal Pradesh, India. *Asian Pac J Trop Dis* 2012; **2**(4): 264-7.

[13] Soulsby EJL. *Helminths, arthropods and protozoa of domesticated animals*. 7th ed. London: Bailliere Tindal; 1982.

[14] Ministry of Agriculture, Fisheries and Food. *Manual of veterinary parasitological laboratory techniques*. London: HMSO; 1986.

[15] Ansari MZ, Rai MK, Chauhan HVS. Pathology of liver, lung and caecum of yak (*Bos poephagus*) infected with *Dicrocoelium*, *Fasciola*, *Echinococcus* and *Trichuris*. *Indian J Anim Sci* 1989; **59**: 552-4.

[16] RangaRao GS, Sharma RL, Hemaprasanth. Parasitic infections of Indian yak *Bos* (*poephagus*) *grunniens*-an overview. *Vet Parasitol* 1994; **53**: 75-82.

[17] Goswami A, Laha R, Sarma D, Chatlod LR. A report on gastrointestinal parasitic infections in yaks. *Indian J Hill Farming* 2013; **26**: 42-4.

[18] Rahman H, Pal P, Bandyopadyay S. Occurrence of gastrointestinal parasites in domestic yaks in Sikkim. *Indian J Anim Sci* 2010; **80**: 195-8.

[19] Bandyopadhyay S, Pal P, Bhattacharya D, Bera AK, Pan D, Rahman H. A report on the prevalence of gastrointestinal parasites in yaks (*Bos poephagus*) in the cold desert area of North Sikkim, India. *Trop Anim Health Prod* 2010; **42**: 119-21.

[20] Weiner G, Jianlin H, Ruijun L. *The yak*. Bangkok: Regional Office for Asia and the Pacific; 2003.

[21] Hogg K. Internal parasites of yak (*Poephagus grunniens*) of the Gannan, Gansu Province, P.R. China. Proceedings of the International Congress on Yak, Chengdu, Sichuan, P.R. China. 2004.

[22] Gielhausen HE. Serological survey on infectious diseases of a white yak herd in the Gansu Province. Proceedings of the 3rd International Congress on Yak; 2000 Sep 4-9; Lhasa. Nairobi: International Livestock Research Institute; 2002.

[23] Degen AA, Kam M, Pandey SB, Upreti CR, Pandey S, Regmi P. Transhumant pastoralism in yak production in the lower Mustang District of Nepal. *Nomad People* 2007; **11**: 57-85.

[24] Acharya KP, Nirmal BK, Poudel B, Bastola S, Mahato MK, Yadav GP, et al. Study on yak husbandry in Mustang district of Nepal. *J Hill Agric* 2014; **5**(2): 100-5.

[25] Acharya KP, Kaphle K. Ethnomedicinal plants used by yak herders for management of health disorders. *J Intercult Ethnopharmacol* 2015; **4**(4): 270-6.