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Prevalence of gastrointestinal parasites in equines of Mustang District, Nepal

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Abstract. *Devkota RP, Subedi JR, Wagley K. 2021. Prevalence of gastrointestinal parasites in equines of Mustang District, Nepal. Biodiversitas 22: 3958-3963.* The study was conducted from March 2018 to November 2018 in four villages of Mustang district to find out the prevalence of gastrointestinal parasites in equines (horses and mules). Fecal samples collected from 175 randomly selected equines (105 from horses and 70 from mules) were preserved in 2.5% potassium dichromate and microscopically examined using concentration techniques to detect ova, larvae, and oocysts of parasites. The overall prevalence of gastrointestinal parasites was found to be 84.57% with an incidence rate of 81.90% and 88.57% in horses and mules respectively. The prevalence of gastrointestinal parasites between horses and mules did not vary significantly ($p>0.05$). Also no significant association ($p>0.05$) between the prevalence rate of parasites and study areas was observed. Among identified parasites in horses, Strongyles showed the highest prevalence (68.57%) followed by *Strongyloides* spp. (23.80%), and *Parascaris equorum* (14.28%) and in mules Strongyles showed the highest prevalence (80.0%) followed by *Parascaris equorum* (35.71%), *Strongyloides* spp. (28.57%), and *Dictyocaulus* spp. (5.71%). Prevalence of gastrointestinal parasites showed no significant variation ($p>0.05$) with respect to age and feeding status. However, the prevalence rate significantly varied ($p<0.05$) with respect to parameters like infection status (single and mixed), housing condition and health status. The present study revealed a higher prevalence rate of gastrointestinal parasites due to poor husbandry practices and lack of adequate veterinary services. Therefore, improvement in housing and feeding system, awareness regarding effective regular deworming, and establishment of veterinary hospitals in the study areas were recommended.

Keywords: Gastrointestinal parasites, horses, mules, Mustang District, prevalence

INTRODUCTION

Equine power is essential for both rural and urban transportation networks since it is inexpensive and provides the greatest option in situations where the road network is inadequate in mountainous regions and towns with narrow streets. Equines play a crucial role in urban and rural areas, providing agricultural energy and transport. In many cases, the sole means of income-generating for their resource-limited owners (Getachew et al. 2014). The equine population in the world is about 122.4 million with 35% being horses, 33% donkeys, 20% zebras and camels, and 12% mules (Ali et al. 2018). Globally 98% of donkeys, 97% of mules, and 60% of horses are concentrated in developing countries (Tedla and Abichu 2018). As per Statistical Information on Nepalese Agriculture, Nepal has a total of 55,808 equine populations with around 2000 equines in Mustang district. There has been a gradual decline in equine population since 1980's due to road construction, reduction in grassland and forest area, and to some extent, availability of air transport. However, mules are still primarily used for domestic good transport in the track of high and mid mountains of Nepal. Most of the mules being used in Nepal are brought from neighboring part of India and only a few of them are native breeds.

Equines harbor a large number of parasites in the Gastrointestinal tract causing varying degrees of damage depending on the age, nutritional and immune status of equines (Asefa et al. 2011). Gastrointestinal helminth infections, particularly nematode and trematode infections, have a significant impact on livestock output by causing death and morbidity and expenses associated with control and treatment (Lashari and Tasawar 2011). Moreover, the infection is associated with loss of digestive function, decreased fertility and poor body condition of animals (Brady and Nichols 2009). Among different groups of the helminths, large strongyles are the most pathogenic gastrointestinal parasites of equines (Sori et al. 2017). A number of studies conducted to detect an association between poverty and animal diseases have identified gastrointestinal parasitism as one of the most important problems for equids in developing countries (Mezgebu et al. 2013).

In Nepal, few studies are available from different parts of the country regarding the prevalence of gastrointestinal parasitism in equines and have reported prevalence ranging from 45% to 100% (Karki and Manandhar 2006; Sapkota 2009). Apart from few reports in other parts of Nepal, there was no specific previous information on intestinal parasites of equines in Mustang district, Nepal. Therefore, this study was carried out in four villages of Mustang district to know the current prevalence of gastrointestinal parasites and the association of prevalence with different risk factors.

MATERIALS AND METHODS

Study area

Mustang District is located in Nepal's Gandaki Province in between 28°33' 51" North to 29°19' 52" North latitude and 83°28' 54" east to 84°14' 58" east longitude. The district stands at the Himalayas and expands northward onto the Tibetan plateau. It is a vast and arid valley, distinguished by eroded canyons, vividly colored stratified rock formations, and barren high-altitude deserts with very sparsely distributed households where the residential area comprises only 0.08% of the total area. The study area receives an average annual rainfall of less than 260 mm. The average minimum monthly air temperature drops down to -2.7°C in winter while the maximum monthly air temperature reaches to 23.1°C in summer. The whole district is located within the Annapurna Conservation Area. The study areas selected included Thini, Muktinath, Surkhang and, Lomanthang of Mustang district, and the altitude of the study area varied from 2,500 m to 3,800 m above sea level.

Study design and samples collection

A total of 175 freshly defecated fecal samples were taken between 4 a.m. to 6 a.m. In this study, only one host animal per dwelling was chosen. Four villages in the Mustang district were chosen to guarantee consistent sampling in order to reflect the entire study area. Each village provided a different number of samples. Of the total samples collected (n =175) horses and mules comprised 105 and 70 samples respectively. Collected fecal samples were kept inside the clean plastic vial and preserved in 2.5% potassium dichromate. Each sample was labeled with its corresponding date, location, species, housing and feeding condition, age and, health status of the host animal. Age of the animals was determined using dentition and information provided by the owner (Mezgebu et al. 2013). Equines below three years were classified as young, those in the range of three to ten years were classified as adults and above ten years were classified as old (Debere et al. 2018). The housing that provided enough resting space, thermal comfort and ease of movement for animals were considered as good housing (Ivet 2020). Whereas, housing where equines couldn't withstand cold weather and heavy rains with lack of proper and sturdy fencing, unclean and wet area for feeding, and where equines could not stand up freely or lie down comfortably were considered poor housing. Similarly, the health status of equines under the study was divided into three categories i.e. healthy, weak and, emaciated on the basis of their physical appearance/body condition (Ali et al. 2018). The imperturbable samples were brought to the laboratory of the Central Department of Zoology, Kirtipur, Kathmandu and were processed after 72 hours as immediate processing was not possible.

Coprological examination

The fecal samples were subjected to coprological examination by concentration techniques (flotation and sedimentation). Approximately 3 g of fecal sample was taken in a beaker and 50 ml of water was poured and mixed thoroughly. The fecal suspension was run through a tea strainer into another beaker. Then the fecal suspension was poured into a centrifuge tube of 15 ml and centrifuged at 2,000 rpm for 5 minutes. The tube's water was replaced with saturated sodium chloride solution and was again centrifuged. More saturated sodium chloride solution was gently filled leaving a convex meniscus surface at the top of the tube and a coverslip was carefully placed on the top of the tube. Then the tube was let to stand for 20 min. The coverslip was removed and placed gently on a clean glass slide and examined under the microscope. After the examination of the flotation portion, the sediment content was poured into the watch glass and stirred gently to mix it. One drop from the mixture was taken on slide and stained with iodine to examine under the microscope. The eggs and larvae present in the positive fecal samples were identified using standard key based on their morphological traits as described by Soulsby (1982). The L3 larvae of *Dictyocaulus* spp. were recovered from mules' fecal samples by Baermanns technique and were identified using a stereomicroscope.

Data management and analysis

The data collected from the study were coded and entered in a Microsoft Excel spreadsheet and, the statistical analysis was performed using SPSS, version 25 software packages. The chi-square test was used to assess the difference in the frequency of gastrointestinal parasites among different variables like equine species, study area, infection status, age, feeding condition, housing condition and health status. The percentage was used to calculate the prevalence rate of gastrointestinal parasites. In all cases, 95% confidence interval (CI) and $p<0.05$ were considered for a statistically significant difference.

RESULTS AND DISCUSSION

An overall 84.57% (148/175) prevalence of gastrointestinal parasites was observed with 81.90% (86/105) and 88.57% (62/70) prevalence in horses and mules respectively, the difference being statically non-significant ($p\geq 0.05$) between horses and mules (Table 1). Among identified parasites in horses, the Strongyle worm eggs showed the highest prevalence (68.57%) followed by *Strongyloides* spp. (23.80%) and *Parascaris equorum* (14.28%). Similarly, in mules the eggs of Strongyles showed the highest prevalence (80%) followed by *Parascaris equorum* (35.71%), *Strongyloides* spp. (28.57%) and larvae of *Dictyocaulus* spp. (5.71%) (Table 2).

Among four study areas, the highest prevalence in horses was revealed from Muktinath followed by Lo-Manthang, Thini and Surkhang with the prevalence of 90.47%, 84.21%, 66.66% and 60.0% respectively. Similarly, the highest prevalence in mules was revealed from Muktinath followed by Lomanthang, Thini and Surkhang with the prevalence of 100%, 93.33%, 87.5% and 81.81% respectively. The study showed no statistically significant difference ($p>0.05$) between the study area and

the prevalence of gastrointestinal parasites in both horses and mules (Table 3).

Both horses and mules were found to be infected with single and mixed gastrointestinal parasites infection. In the current study, single infection was observed in 60.95% of horses and 38.57% of mules (Table 4) while the mixed infection was found in 20.95% of horses and 50% of mules (Table 5) and a significant association ($p<0.05$) was observed between the infection status (single and mixed) and prevalence of gastrointestinal parasites among horses and mules (Tables 4 and 5). The data were further analyzed with respect to different risk factors such as age, feeding status, housing condition and, health status of equines. The prevalence in young equines was 93.33% while the prevalence in adult and old equines was 83.05% and 85.71% respectively. A higher prevalence (88.57%) was observed among equines that graze on pastures while poor housing conditions contributed to the prevalence of 89.32%. Based on the health status of equines the prevalence of gastrointestinal parasites was 100% in emaciated equines, 91.30% in weak and 79.46% in strong equines Prevalence of gastrointestinal parasites showed no significant difference ($p>0.05$) with respect to age and feeding status. However, the prevalence rate was significantly associated ($p<0.05$) with housing conditions and health status (Table 6).

Table 1. Overall Prevalence of gastrointestinal parasites in equines

Equines	No. of animal examined	No. of animal positive	Prevalence (%)	χ^2	p-value
Horse	105	86	81.90	13.385	0.342[#]
Mule	70	62	88.57		
Total	175	148	84.57		

Note: [#]: Statically non-significant

Table 2. Types of gastrointestinal parasites observed in horses and mules on coprological examination

Egg/larvae of parasite	Horse			Mule		
	Total examined	Positive	Prevalence (%)	Total examined	Positive	Prevalence (%)
Strongyles	105	72	68.57	70	56	80
Strongyloides spp.	105	25	23.80	70	20	28.57
Parascaris equorum	105	15	14.28	70	25	35.71
Dictyocaulus spp.	105	0	0	70	4	5.71
Total	105	86	81.90	70	62	88.57

Table 3. Area wise prevalence of gastrointestinal parasites in horses and mules

Study Location	Horse			Mule		
	Total examined	Positive n(%)	χ^2 (p-value)	Total examined	Positive n(%)	χ^2 (p-value)
Thini	15	10(66.66)	7.80	24	21(87.5)	2.51
Muktinath	42	38(90.47)	(0.052)[#]	9	9(100)	(0.472)[#]
Surkhang	10	6(60)		22	18(81.81)	
Lo-Manthang	38	32(84.21)		15	14(93.33)	
Total	105	86(81.90)		70	62(88.57)	

Note: [#]: Statically non significant

Table 4. Prevalence of single infection of gastrointestinal parasites in horses and mules

Egg/larvae of parasite	Horse		Mule	
	No. of positive	Prevalence (%)	No. of positive	Prevalence (%)
Strongyles	50	47.61	22	31.42
Strongyloides spp.	9	8.57	2	2.85
Parascaris equorum	5	4.76	3	4.28
Dictyocaulus spp.	0	0	0	0
Total	64	60.95	27	38.57

Table 5. Prevalence of mixed infection of gastrointestinal parasites in horses and mules

Egg/larvae of parasite	Horse		Mule	
	No. of positive	Prevalence (%)	No. of positive	Prevalence (%)
Strongyles and *Strongyloides* spp.	13	12.38	9	12.85
Strongyles and *Parascaris equorum*	7	6.66	13	18.57
Strongyloides spp. and *Parascaris equorum*	0	0	1	1.42
Strongyles and *Dictyocaulus* spp.	0	0	4	5.71
Strongyles, *Strongyloides* spp. and *Parascaris equorum*	3	2.85	8	11.42
Total	22	20.95	35	50

Table 6. The prevalence of gastrointestinal parasites with respective categories of the risk factors in the study area

Risk factor	No. of animal examined	No. of animal positive	Prevalence (%)	χ^2	p-value
Age					
Young	15	14	93.33	1.13	0.567#
Adult	118	98	83.05		
Old	42	36	85.71		
Feeding					
Pasture	70	62	88.57	1.55	0.458#
Mixed	96	79	82.29		
Grain	9	7	77.77		
Housing					
Good	72	56	77.77	4.32	0.037*
Poor	103	92	89.32		
Health status					
Healthy	112	89	79.46	6.93	0.031*

Note: *: Statically Significant #: Statically non-significant

Discussion

The coprological examination done for the current study using floatation and sedimentation techniques revealed an overall gastrointestinal parasites prevalence to be 84.57% which is higher than earlier reports of Singh et al. (2012), Goraya et al. (2013), Matto et al. (2015), and Ali et al. (2018) who reported the prevalence rates to be 20.63%, 32.2%, 72% and 74.06% in organized farms of Mumbai and Pune, Upper Punjab of Pakistan, Bajaur and Mohmand agencies of Northwest Pakistan and Jammu region of India respectively. High prevalence reported might be due to variation in sampling time as seasonality affects the occurrence of the parasites, feeding practice and, deworming habit (Regassa and Yimer 2013). In this study, the prevalence of 88.57% and 81.90% was recorded in mules and horses respectively the difference being statically non-significant ($p>0.05$). This is higher than the study of Sapkota (2009), Matto et al. (2015), and Tahir et al. (2016) who reported the prevalence of 14.80%, 43.79%, and 45% in Mumbai & Pune of India, Faisalabad region of Pakistan and Lalitpur district of Nepal respectively, but lower than the Karki and Manandhar (2006) who reported the prevalence rate of 100% in Udayapur district of Nepal. Similarly, the prevalence rate in horses (81.90%) observed in the present study almost correlates with the study carried by Adeppa et al. (2016) in the Shimoga region of India and Oli and Subedi (2018) in Rukum District of Nepal who reported the prevalence of 84% and 84.76% respectively, but is lower than the findings of Berhanu et al. (2014) and Hasson (2014) who reported an overall prevalence of 97.9% and 100% in Hawassa Town Ethiopia and Baquba city Iraq respectively. The present findings is higher than the reports of Yadav et al. (2014), Matto et al. (2015), Tahir et al. (2016), Ali et al. (2018), and Khan et al. (2020) who reported the prevalence rates of 38.69%, 38.79%, 43.33%, 44.6% and 59.25% in Faisalabad region of Pakistan, Organized farms of Mumbai and Pune, Government farm Sargodha of Pakistan, Bajaur and Mohmand agencies of Northwest Pakistan and Jabalpur of North India respectively. The variation in results regarding workers from different regions might be due to variation in the management system, geographical and climatic condition, sample size, sample collection period, and sampling method differences (Oli and Subedi 2018). The higher prevalence of gastrointestinal parasites in both horses and, mules could be due to poor feeding practice, high exposure in pasture grazing, inadequate resting time, excessive work burden and, less attention from the owners of the study area.

The highest prevalence of Strongyles in both species (80% in mules and 68.57% in horses) is almost in accordance with the studies of Umar et al. (2013) and Kachhawa et al. (2015) in horses of Nigeria and Bikaner Rajasthan of India who reported prevalence of 68.8% and 68.18% respectively. The prevalence of Strongyles in the current study is higher than 10.01%, 10.81%, and 14.90% as reported by Pilania et al. (2012), Matto et al. (2015), and Tahir et al. (2016) in equines of Rajsthan, organized farms of Mumbai & Pune and Faisalabad region of Pakistan respectively, but is lower than 100% prevalence reported by Karki and Manandhar (2006) in mules from Udayapur district of Nepal. Similarly, the prevalence of *Strongyloides* spp. reported to be 23.80% in horses and 28.57% in mules is in agreement with the previous reports of 22.72% and 28.4% prevalence in equines from Hawasa town Ethiopia and Al Diwaniyah Governorate Iraq as reported by Wannas (2012) and Berhanu et al. (2014) respectively. Prevalence of *Strongyloides* spp. reported in the present study is higher than earlier reports of Maria et al. (2012), Matto et al. (2015) and Ali et al. (2018) who reported prevalences of 13.19%, 6.90%, and 0.14% in Mumbai and Pune, the central zone of Kashmir valley and Northwest Pakistan respectively.

The prevalence rate of *Parascaris equorum* in horses was 14.28% which is closely related to prevalence of 15.51% reported by Sultan et al. (2014) from Kurfa Chale Ethiopia, but is lower than prevalence (33.33%) reported by Khan et al. (2020) from Sargodha Pakistan and higher than 7.09% and 10.47% prevalence reported by Pilania et al. (2012) and Oli and Subedi (2018) from horses of Rajasthan India and Rukum district of Nepal respectively. Similarly, the prevalence rate of *Parascaris equorum* in mules was 35.71% which correlates with the prevalence of 34.60% reported from equines by Tahir et al. (2016) in Faisalabad region of Pakistan but lower than prevalence (50%) reported by Karki and Manandhar (2006) in mules of Udayapur district Nepal. The present findings are higher than the findings of Matto et al. (2015) who reported prevalence of 0.23% in equines from organized farms of Mumbai & Pune. The difference in the prevalence of *Parascaris equorum* from other workers could be due to compromised immune responses related to concurrent diseases (Upjohn et al. 2010). Moreover, the prevalence of 5.71% of larvae of *Dictyocaulus* spp. recorded only from mules in the present study almost correlates with the study carried out at brick kiln of Lalitpur Nepal by Sapkota (2009), but is higher than previous studies of Saeed et al. (2010), Tolossa and Ashenafi (2013), and Matto et al. (2015) who reported prevalence of 0.5%, 2.5%, and 0.23%

respectively. The reports of Umar et al. (2013) and Ali et al. (2018) with prevalence of 10.4% and 13.62% is higher than the prevalence of *Dictyocaulus* spp. reported in the present study. Climatic and environmental differences and differences in access to drugs may partly explain the variation (Mezgebu et al. 2013; Adeppa et al. 2016).

Regarding the risk factor analysis, the current study reported non-significant association between species, study areas, age, and feeding practice and prevalence of equine gastrointestinal parasites while significant statistical difference was observed between the prevalence of equine gastrointestinal parasites and infection status, housing condition and health status. The variations in prevalence rate among four villages could be mainly due to altitudinal and habitat variation (Oli and Subedi 2018). The upland of cooler areas and habitat near river or water origin had more prevalence than lowland (Courtney 1999). Overall 32.57% of positive samples had mixed infection with the highest prevalence shown by Strongyles and *Strongyloides* spp. (12.38%) in horses and Strongyles *and Parascaris equorum* (18.57%) in mules. Mixed infection observed in the current study is higher than the previous findings of Singh et al. (2012), Matto et al. (2015), and Khan et al. (2020) who reported the prevalence of 5.44%, 10.26%, and 12.43 % in organized farms of Mumbai and Pune, Sargodha Pakistan, and Jammu region of India respectively. Higher mixed infection in equines could be due to lack of de-worming programs, very less use of anti-parasitic drugs, dietary deficiency and poor management system (Saeed et al. 2010; Mezgebu et al. 2013).

The prevalence in young equines was 93.33% while the prevalence in adult and old equines was 83.05% and 85.71% respectively which is higher than the findings of Tesfu et al. (2014) and Taye (2017) of Mekelle and Hawasaa town of Ethiopia respectively. Strongyles eggs predominated in all the infected young equines which could be due to lack of immunity in the younger population (Taye 2017). On contrary to our report, a higher prevalence rate among old equines (>10 years) has been reported by different studies attributing it to deteriorating body condition and senile immune suppression (Sapkota 2009; Ali et al. 2018). Several previous epidemiological investigations have found that young horses are more susceptible to nematode infection than older horses (Saeed et al. 2010; Tesfu et al. 2014). A Higher prevalence (88.57%) was observed among equines that graze on pastures. This could be due to the fact that grazing animals are more likely to be exposed to parasites from the grass (Taye 2017). Poor housing condition was another factor that contributed to acquiring gastrointestinal parasites in equines as higher prevalence of 89.32% was observed which correlates with the previous finding of Mezgebu et al. (2013) at Gondar Town Ethiopia. Based on the health status of equines, the prevalence of gastrointestinal parasites was 100% in emaciated equines, 91.30% in weak, and 79.46% in strong equines which is in line with the study of Ali et al. (2018) who reported 100% prevalence in emaciated equines at Mohmand and Bajaur Agencies of North-West Pakistan. This might be because of the result of parasitosis on animals (Taye 2017).

In conclusion, the study carried out in four villages of Mustang district revealed higher occurrence of gastrointestinal parasites with an overall prevalence of 84.57%. The prevalence was found to be 81.90% and 88.57% in horses and mules respectively. The identified gastrointestinal parasites include eggs of Strongyles, *Strongyloides* spp., *Parascaris equorum* and larvae of *Dictyocaulus* spp. The highest prevalence of Strongyles in both species equines. The prevalence of equine's gastrointestinal parasites showed no significant variation with respect to parameters like host's species, age, study area and feeding status. However, prevalence rate was significantly associated with housing condition, infection status, and health status, which were important risk factors for equine gastrointestinal parasitism in the study area.

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