

Studies on gastro-intestinal helminths of *Equus acinus* in North Gujarat, India

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Abstract

A year round study (October to September) was conducted in the districts of North Gujarat (India) to identify the gastro-intestinal helminths of donkeys (*Equus acinus*), determine prevalence and correlate haematological parameters with parasite burdens. A total of 1794 faecal samples of donkeys contained the following helminths (prevalence % in brackets): *Strongyloides westeri* (17.2), *Parascaris equorum* (23.8), *Strongylus* sp. (55.3), an amphistome digenean (1.5), *Anoplocephala* sp. (1.0), *Balantidium coli* (13.1) and *Eimeria leuckarti* (7.0). Overall prevalence was 75.9 with a mean of 627 (50-1650) eggs per gram faeces. Seasonally the maximum prevalence (85.3) occurred in March and minimum (65.2) in July. 14% of donkeys were considered to be severely infected, 38% heavily, 36% moderately and 12% mildly infected. Larval cultures revealed the presence of (prevalence%): *Cyathostomum* sp. (48), *Gyalocephalus* sp. (8), *Oesophagodontus* sp. (6), *Triodontophorus* sp. (10), *Strongyloides westeri* (10), *Strongylus vulgaris* (30), *Strongylus equines* (40) and *S. edentatus* (30). Hematological indices were inversely proportional to egg counts.

Keywords: donkeys, gastro-intestinal parasites, haematology, prevalence.

Introduction

The donkey (*Equus acinus*) has been a beast of burden for thousands of years, often as a creature of abject slavery, overladen, underfed and ill-used: "to the smallest ass shall go the biggest stick." However, in spite of mechanization throughout the world, the donkey is one of the most under-appreciated but important draught animal, serving a key role in the agricultural economy of the developing world. It is estimated that 50 per cent of the energy required for agricultural production in the world is derived from animals, and the donkey is a major contributor to this need.

Donkeys need little attention and small quantities of food to provide sustained work on poor forage with little rest. As a result, they are considered to be excellent pack animals for transporting heavy loads in the hills, desert and plain areas in countries such as India, Egypt, Sudan, Somalia, Persia and China. In tropical countries such as India, they are the cheapest and easiest means of transport, suiting needs of washer man, potters, house builders, brick manufactures and Vanjara engaged in earth work till today (Mwenya & Tandkeib 2004). There are about 40 million donkeys in developing countries, the full potential use of which still remains to be exploited. According to FAO (2002) census, India's donkey population is 300,000, constituting 2.25% of the total world population. The donkey population of Gujarat is about 80,155 constituting 27% of the Indian donkey population. Thus donkeys contribute immensely in the overall economic development of India, especially in rural areas of Gujarat.

In spite of their paramount economic importance, due attention has not been paid to the conservation of Indian donkey breeds and to upgrade them by undertaking breeding programmes and health management. Even today the stoic, hardworking donkey is often misunderstood by its owner and unfortunately often by veterinarians worldwide. Being very

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poor, donkey keepers often have a myopic attitude regarding the health of their animals. This has resulted into considerable reduction in their work output, reproductive performance and longevity. Donkeys are vulnerable to an array of diseases of biological origin: parasitic, bacterial, fungal and viral diseases. Among parasitic diseases, babesiosis, trypanosomiasis and gastro-intestinal strongylosis are considered to be silent killers (Mekebib *et al.* 2010). Parasitic infections are common and varied, exercising a great influence on the performance of donkeys.

The attention given by governmental and non-governmental organizations to donkeys has been far below what they deserve. Despite their huge numbers and increasing importance in the Indian economy, knowledge about the health problems affecting the welfare of donkeys is limited for most parts of the country. Therefore the objectives of this study were to determine the spectrum of species and prevalence of the major gastro-intestinal parasites of donkeys, their seasonal pattern and to test for their association with measurable haematological parameters.

Materials & Methods

The study was conducted from October to the following September in the Banaskantha, Sabarkantha, Mehsana and Patan districts of North Gujarat, India (20° to 35° N, 71 ° to 73° E), within the tropical region where the climate is arid to semi-arid, with less precipitation than other areas of Gujarat. In North Gujarat, summer starts in March/April and ends in June, followed by the south-western monsoon. The extreme conditions rise above 40°C in summer, and fall to 5 °C in winter. The long rainy season is between June and October, with an average rainfall of 480 mm.

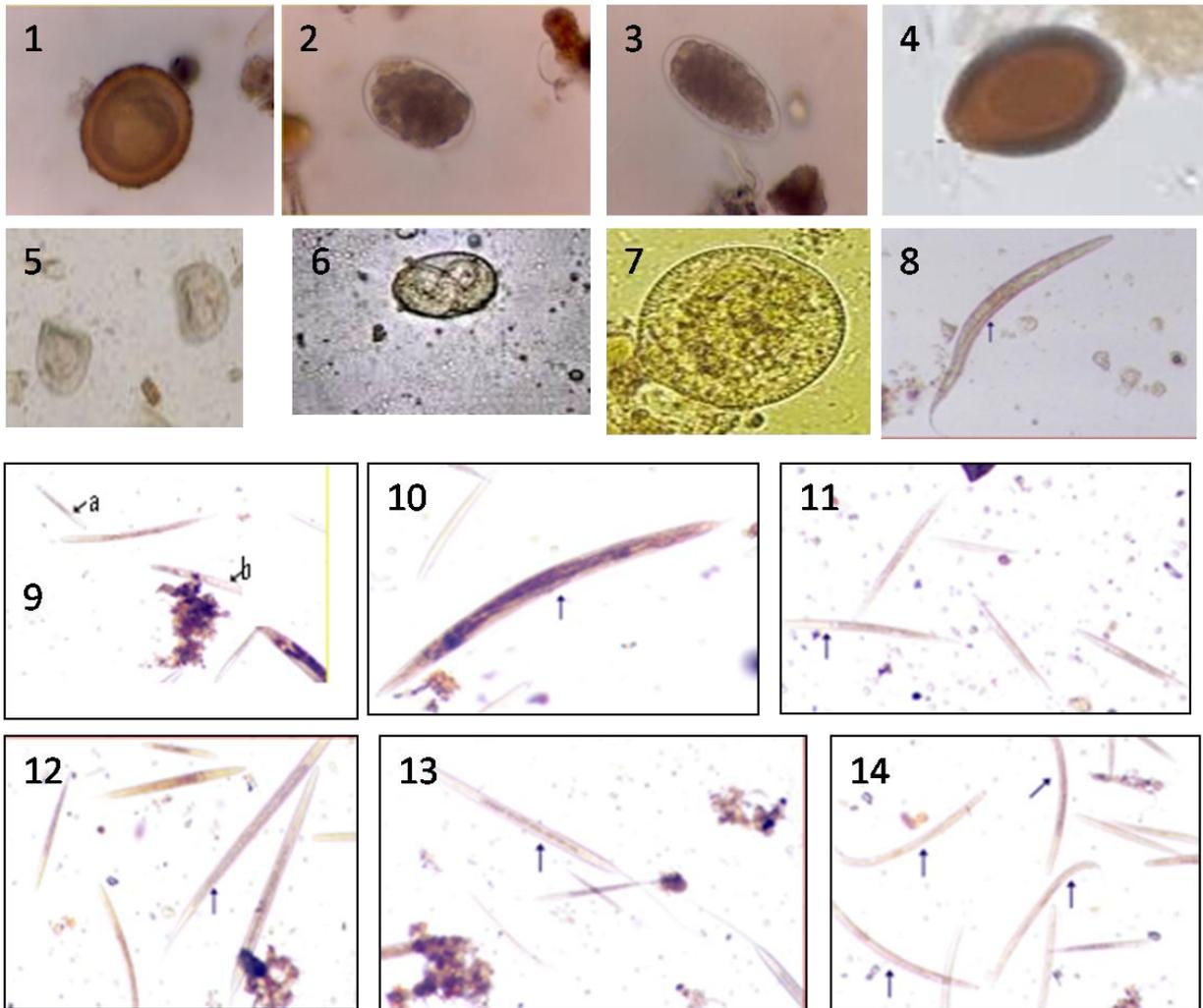
Feeding and management practices are not established for domesticated donkeys, so they were kept solely on grazing with no extra fodder provided. No de-worming or vaccination was carried out during the study period.

A total of 1794 faecal samples were collected and subjected to quantitative and qualitative coprological examination to identify the major gastro-intestinal parasites involved, determine their prevalence and find out their association with haematological parameters. The donkey keepers selected for the study were informed of the importance of the study, and asked to present their donkeys on specific visit dates and places. Faecal samples were taken directly from the rectum or the ground when the animal was seen defecating. Under strict sanitation, samples were placed in air- and water-tight vials and brought to the laboratory. Gross faecal examinations were carried out before being subjected to detailed examination under the microscope. The modified McMaster and Bearman techniques, and sedimentation and floatation methods, were used to identify and count the helminth eggs and larvae (Urquhart *et al.* 1996). Faecal cultures (n=100) were carried out for samples with total mean egg counts greater than 500. Identification of larvae (L₃) was based on specific morphological traits. Levels of worm infection were extrapolated from the infection severity index defined by Soulsby (1986) where horses were said to have mild, moderate, heavy and severe nematode infestations if their faecal egg counts were less than 500, 800-1000, 1100-1500 and more than 1500, respectively.

Blood samples were collected aseptically from the jugular vein with the help of vacuette needles (20G). Nine ml of blood was drawn into each vacuette EDTA (Greinerbio-one), labelled and transported to the laboratory in an icebox. Haematological analyses comprised of haemoglobin, total erythrocyte count, total leucocyte count, packed cell volume, erythrocyte sedimentation rate and differential leucocyte count, and were carried out according to the method described by Jain (1986). The data were analyzed using Student's t- test as described by Snedecor & Cochran (1989).

Results

The prevalence of gastro-intestinal helminthic infection in donkeys was almost 76%, with a mean of 627 (range 50-1650) eggs per gram of faeces (Table 1), and was similar in all the districts of North Gujarat studied. Overall monthly prevalence reached a maximum in March and minimum in July (Table 1). The species found were *Strongyloides westeri* (Fig. 6), *Parascaris equorum* (Fig. 1), *Strongylus* sp. (Figs. 2 & 3), an unidentified amphistome digenean, *Anoplocephala* sp. (Fig. 5), *Balantidium coli* (Fig. 7) and *Eimeria leuckarti* (Fig. 4): their prevalences are given in Table 1. Comparatively greater helminth prevalence was recorded in the period January to April than in May to July. The greatest overall prevalence was of strongylosis at a maximum of 55.3% (Table 1).



Figures: 1) ova of *Parascaris equorum*; 2) ova of *Strongylus* sp.; 3) ova of *Strongylus* sp.; 4) oocyst of *Eimeria leuckarti*; 5) ova of *Anoplocephala* sp.; 6) ova of *Strongyloides westeri*; 7) cyst of *Balantidium coli*; 8) larvae of *Cyathostome* sp.; 9) larvae of (a) *Gyalocephalus* sp. and (b) *Oesophagodontus* sp.; 10) larvae of *Triodontophorus* sp.; 11) larvae of *Strongyloides westeri*; 12) larvae of *Strongylus vulgaris*; 13) larvae of *Strongylus equinus*; 14) larvae of *Strongylus edentatus*.

Month	N	positives (%)	Mean EPG (range)	Species detected (prevalence %)
June	150	104 (69.3)	513.6 (100 - 1150)	<i>Strongyloides</i> (25), <i>Parascaris equorum</i> (29), <i>Strongylus</i> (71), <i>Balantidium coli</i> (23), <i>Eimeria leuckarti</i> (8), amphistome (3), <i>Anoplocephala</i> sp. (2).
July	155	101 (65.2)	572.1 (50 - 1150)	<i>Strongyloides</i> (21), <i>Parascaris equorum</i> (30), <i>Strongylus</i> (71), amphistome (4), <i>Balantidium coli</i> (21), <i>Eimeria leuckarti</i> (14), <i>Anoplocephala</i> sp. (1)
August	157	112 (71.3)	646.5 (50 - 1450)	<i>Strongyloides</i> (24), <i>Parascaris equorum</i> (35), <i>Strongylus</i> (80), <i>Balantidium coli</i> (23), <i>Eimeria leuckarti</i> (11), <i>Anoplocephala</i> sp. (2), amphistome (2)
September	150	124 (82.7)	688.3 (150 - 1450)	<i>Strongyloides</i> (30), <i>Parascaris equorum</i> (41), <i>Strongylus</i> (92), <i>Balantidium coli</i> (22), <i>Eimeria leuckarti</i> (14), amphistome (3)
October	146	115 (78.8)	635.4 (100 - 1650)	<i>Strongyloides</i> (26), <i>Parascaris equorum</i> (9), <i>Strongylus</i> (23), <i>Balantidium coli</i> (4), <i>Eimeria leuckarti</i> (1), <i>Anoplocephala</i> sp. (1)
November	145	102 (70.3)	563.1 (100 - 1250)	<i>Strongyloides</i> (24), <i>Parascaris equorum</i> (32), <i>Strongylus</i> (76), <i>Balantidium coli</i> (15), <i>Eimeria leuckarti</i> (6), amphistome (3)
December	146	103 (70.6)	511.8 (100 - 1250)	<i>Strongyloides</i> (22), <i>Parascaris equorum</i> (35), <i>Strongylus</i> (78), <i>Balantidium coli</i> (14), <i>Eimeria leuckarti</i> (9), amphistome (2), <i>Anoplocephala</i> sp. (1)
January	140	114 (81.4)	683.8 (50 - 1650)	<i>Strongyloides</i> (29), <i>Parascaris equorum</i> (38), <i>Strongylus</i> (80), <i>Anoplocephala</i> sp. (3), <i>Balantidium coli</i> (15), <i>Eimeria leuckarti</i> (8), amphistome (2)
February	149	125 (83.9)	682.3 (100 - 1500)	<i>Strongyloides</i> (28), <i>Parascaris equorum</i> (43), <i>Strongylus</i> (94), <i>Eimeria leuckarti</i> (12), <i>Anoplocephala</i> sp. (2), <i>Balantidium coli</i> (25), amphistome (2)
March	163	139 (85.3)	736.8 (150 - 1650)	<i>Strongyloides</i> (34), <i>Parascaris equorum</i> (40), <i>Strongylus</i> (98), <i>Balantidium coli</i> (22), <i>Eimeria leuckarti</i> (11), <i>Anoplocephala</i> sp. (3), amphistome (3)
April	147	123 (83.7)	746.6 (50 - 1600)	<i>Strongyloides</i> (24), <i>Parascaris equorum</i> (37), <i>Strongylus</i> (87), <i>Eimeria leuckarti</i> (13), <i>Anoplocephala</i> sp. (1), <i>Balantidium coli</i> (24), amphistome (1)
May	146	100 (68.5)	539.3 (100 - 1000)	<i>Strongyloides</i> (22), <i>Parascaris equorum</i> (30), <i>Strongylus</i> (79), <i>Balantidium coli</i> (17), <i>Eimeria leuckarti</i> (10), amphistome (2)
Overall	1794	1362 (75.9)	626.6 (50 - 1650)	<i>Strongyloides</i> (17.2), <i>Parascaris equorum</i> (23.8), <i>Strongylus</i> (55.3), <i>Eimeria leuckarti</i> (7.0), <i>Anoplocephala</i> sp. (1.0), <i>Balantidium coli</i> (13.1), amphistome (1.5)

Table 1: Occurrence and prevalence of helminthic infections in the donkeys of North Gujarat each month through the year: ‘amphistome’ means a digenean with an oral sucker and a posterior acetabulum.

In the faecal cultures, eight remarkably different types of larval helminth parasites were identified, with both large and small strongyles almost equally prevalent in all districts: *Cyathostomum* sp. (48%) (Fig. 8), *Gyalocephalus* sp. (8%) (Fig. 9a), *Oesophagodontus* sp. (6%) (Fig. 9b), *Triodontophorus* sp. (10%) (Fig. 10), *Strongyloides westeri* (10%) (Fig. 11),

Strongylus vulgaris (30%) (Fig. 12), *Strongylus equinus* (40%) (Fig. 13) and *Strongylus edentatus* (30%) (Fig. 14).

The haematological profiles of three groups of donkeys were studied: uninfected (n = 50); and infected (n=50), divided into those with less than 500 eggs per gram faeces, and those with more than 500 eggs per gram (Table 2). There was a significant decrease in mean haemoglobin concentration with greater severity of infection, and a similar declines in the mean total erythrocyte count and packed cell volume (Table 2). There were other apparent increases and decreases between groups, but none were statistically significant.

Parameter	Uninfected	Infected	
		< 500 epg	> 500 epg
Haemoglobin concentration (g dl ⁻¹)	11.4 ± 0.2 ^a	9.3 ± 0.1 ^b	7.7 ± 0.2 ^c
Total leucocyte count	13454 ± 480	13264 ± 454	13856 ± 726
Total erythrocyte count (10 ⁶ kl ⁻¹)	5.2 ± 0.1 ^a	4.7 ± 0.05 ^b	4.1 ± 0.1 ^c
Erythrocyte sedimentation rate	84.2 ± 5.7	93.6 ± 7.5	107.8 ± 7.9
Packed cell volume (%)	40.5 ± 0.4 ^a	34.5 ± 0.2 ^b	29.3 ± 1.0 ^c
Neutrophils	55.0 ± 1.8	53.8 ± 1.7	56.5 ± 2.2
Lymphocytes	38.9 ± 1.7	39.5 ± 1.8	36.9 ± 2.2
Eosinophils	5.6 ± 0.5	5.8 ± 0.7	6.1 ± 0.8
Monocytes	0.58 ± 0.11	0.61 ± 0.07	0.50 ± 0.13

Table 2: Haematological values in uninfected and infected animals divided in low and high infections according to the number of eggs per gram of faeces (epg). Superscript letters denote means that are significantly different (p<0.05) across a row.

Discussion

While gross faecal examinations showed no evidence of gastro-intestinal parasites, microscopic examinations showed that gastro-intestinal parasitosis was actually an important disease in the study area, with an overall prevalence of more than 75%. Based on the severity index defined by Soulsby (1986), 14% of the donkeys were severely infected, 38% heavily infected, 36% moderately infected and 12% mildly infected. All districts showed the same prevalences. Others have also reported the prevalence of helminthic infections in donkeys of between 73% and 90% in India (Pal 2002, Getachew *et al.* 2011), and from 48% to 100% abroad (Ayele *et al.* 2006). Our quantitative levels of infection closely resemble those of Chander & Kumar (2000), who reported 90% prevalence with mean epg of 580 ± 38 in Hisar, India. Maintenance of the high infection rate in North Gujarat may be associated with the lack of any intervention programme, coupled with the grazing management system whereby donkeys are allowed to graze together on small plots of land throughout the year, facilitating contamination between animals.

The prevalence of strongyle infections was 55%, almost the same in the four districts of North Gujarat. Other workers have reported between 8% and 89% prevalence of *Strongylus* spp. from India (Pal 2002), and similar high levels abroad (Ayele *et al.* 2006). From the seasonal variation of strongyle infestation, treatment schedules that enable the elimination of the parasites at peak infection and prevent re-infection of pastures can be proposed. Troney (1989) has proposed two treatment schedules per year with broad-spectrum anthelmintics for tropical conditions. A treatment may be given at the end of the rainy season. At this time, the animals are well-nourished and may harbour large number of parasites without being seriously

affected. Eliminating these parasites will improve the adaptation of the animals to the harsh dry-season conditions. Another treatment can be given at the end of the dry season. This treatment reduces infestation of pastures at the time of first rains by residual parasites.

The prevalence of *Parascaris equorum* was about 24%, similar to earlier reports (Lyons *et al.* 1984, Bhat & Sharma 1990, Chander & Kumar 2000) of 18 - 31%. However, Wadhwa *et al.* (1993) has reported much lower prevalences of 4 - 17% in Himachal Pradesh, but used a smaller sample size. Levels of infestation did not vary seasonally.

The prevalence of *Strongyloides westeri* (17%) is about the same as in Chander & Kumar (2000), but lower than the figures (28-38%) of Ayele *et al.* (2006). The low prevalence in our study area may be the effect of its relatively higher temperatures, which desiccate the highly susceptible eggs and larvae of *Strongyloides westeri*.

The prevalence of the amphistome digenean and *Anoplocephala sp.* were low, in the later case perhaps due to the seasonality of its oribatid mite vectors (Soulsby 1986). The low prevalence of the amphistome digenean might be due to differences in ecological preference for the development of the snail intermediate hosts and the parasite. Wadhwa *et al.* (1993) also reported similarly low prevalences in Himachal Pradesh, but in Haryana much higher prevalences (7 - 31%) have been reported (Sengupta & Yadav 1998) due to the snail population and favourable environmental conditions in the northern part of the country. Our estimates for *Anoplocephala sp.* are similar to those of Sengupta & Yadav (1998), but much lower than some others (Lyons *et al.* 1984, 2000, Pearson *et al.* 1993, Fogarty *et al.* 1994), where levels reach 80%, perhaps because of the availability of the intermediate oribatid mite host in favourable environmental conditions. The prevalences of *Balantidium coli* and *Eimeria leuckarti* were similar to those of Sengupta & Yadav (1998, 2003).

In this study, another striking feature was the low prevalence (55%) of large strongyles. Amongst various possible factors, adverse environmental conditions for survival of the extrinsic stages of the parasite as well as the inability of large strongyles to undergo inhibition inside the host and their long generation interval might be responsible for such low prevalence (Pal 2002). The degree of infection and prevalence were similar in all four districts of North Gujarat, and there was little seasonal fluctuation but infection loads were reduced in dry conditions. Thus we attribute the low prevalence to the unfavorable environmental conditions prevailing in North Gujarat, which experiences intermittent low rainfall, and high humidity as well as temperature throughout the year. This can further be appreciated by comparison of the meteorological data and the percent positive animals studied in the four districts.

The overall seasonal prevalence (Table 1) of helminthic infection, with a maximum in March and minimum in July, was similar in the four districts. The maximum might be caused by the resumption of development of the hypobiotic larvae (L₄) after the chill of winter. This view is supported by Pal (2002) and Mulate (2005), who indicated that faecal egg counts begin to rise to severe levels during the wet season. This could be because of the long pre-patent period of strongyles, which ensures that larvae acquired in one grazing season only reach maturity during the next (Urquhart *et al.* 1996). The lowest intensity of infection during May to July was due to larval inhibition because of unfavourable climatic conditions outside the host (Pal 2002, Ayele *et al.* (2006).

The significant reductions in haemoglobin, total erythrocyte count and packed cell volume indicate normocytic normochromic anaemia, perhaps due to blood loss from ingestion of erythrocytes by the immature *Strongylus sp.* and probably the reduced haemopoiesis as a result of poor metabolism and poor nutrition status. Haematological changes from direct loss of whole blood by nematodes has been postulated for sheep and other animals (Soulsby 1986). Although non-significant here, in other studies increases in the erythrocyte sedimentation rate have been associated with extensive tissue damage in the intestine and liver caused by larval migration, shown in donkeys (Varshney & Uppal 1992), camels (Suchitra Sena *et al.* 2000), cattle (Rajkhowa *et al.* 2004) and sheep (Padmaja *et al.* 2006).

Further study of donkeys aimed at early detection of parasitic infection, or better management and control of parasitic infections, is clearly required. Studies are also required on the impact of parasitic infections on the health status, working efficiency, reproductive efficiency, draftability and longevity of donkeys. Periodic faecal examination and deworming should be carried out at regular intervals to control parasitic infections for better improvement of health status of donkeys, and for the welfare of nomadic tribes that rely on donkeys. Governmental or non-profit development agencies should include donkeys in their priorities for research to develop strategies for sustainable and integrated disease prevention and control programs that are practical for developing communities.

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الملخص العربي

دراسات على الديدان المعوية والمعدية في أحد أنواع الحمير (إيكيس أسنيس) في شمال جيشارات - الهند
حسين بارساني 1 - مؤمن ر. ر. 1 - لطيف أ. 2 - هيمين داس 2

- 1- قسم الطفيليات - كلية الطب البيطري ورعاية الحيوان - جامعة سارداركريشناجار دانتوادا للزراعة - الهند
- 2- قسم بيوكيميائية وفسولوجية الحيوان - كلية الطب البيطري ورعاية الحيوان - جامعة سارداركريشناجار دانتوادا للزراعة - الهند

امتدت الدراسة الحالية لمدة عام كامل (من أكتوبر حتى سبتمبر) في مناطق عديدة بشمال جيشارات بالهند بهدف التعرف على الديدان المعوية والمعدية في أحد أنواع الحمير (إيكيس أسنيس) وذلك بهدف تحديد مدى الإصابة وتأثيرها على خلايا الدم. لقد تم فحص إجمالي 1794 عينة من روث تلك الحمير والتي احتوت على النسب التالية من الطفيليات:

***Strongyloides westeri* (17.2%), *Parascaris equorum* (23.8%), *Strongylus sp.* (55.3%), *amphistome digenean* (1.5%), *Anoplocephala sp.* (1.0%), *Balantidium coli* (13.1%) and *Eimeria leuckarti* (7.0%).**

وكان المدى الإجمالي للإصابة هو 75.9% بمتوسط حسابي قدره 627 (50 - 1650) بيضة في كل جرام من روث الحيوان. وكان معدل الإصابة مرتفعاً في شهر مارس بنسبة 85.3%، ومنتدي في شهر يوليو بنسبة 65.2%. كانت نسبة الإصابة الحادة بالحيوانات تمثل حوالي 14% من مجموعة الحيوانات التي تم فحصها، وكان هناك حوالي 36% ذات إصابة متوسطة، ونسبة 12% ذات إصابة ضعيفة. وعند إجراء مزرعة ليرقات الديدان كانت نسب الإصابة كالتالي:

***Cyathostomum sp.* (48%), *Gyalocephalus sp.* (8%), *Oesophagodontus sp.* (6%), *Triodontophorus sp.* (10%), *Strongyloides westeri* (10%), *Strongylus vulgaris* (30%), *Strongylus equines* (40%) and *S. edentatus* (30%).**

أيضاً أوضحت الدراسة أن تحليل خلايا الدم أوضحت تناسباً طردياً مع معدلات الإصابة.