

Developing a Practical and Reliable Protocol to Assess Nematode Infections in Asian Elephants

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Abstract. Nematodes are a leading cause of economic loss and morbidity in livestock and also one of the more commonly assessed internal parasite groups in domestic animals. Nematode infections may also occur in wild animals including elephants. Current methods of assessing nematode infections suffer from a number of drawbacks in application to free ranging elephants. Here we describe a new method of quantifying nematode infections, based on faecal culture of nematode eggs and harvesting through Berman technique, that is robust, easy to conduct and overcomes many of the problems with other techniques.

Introduction

Animal parasites are organisms that live in (internal or endoparasites) or on (external or ectoparasites) a host-animal. Internal parasites are of particular importance in the case of free ranging Asian elephants (*Elephas maximus*) as management actions that confine elephants to restricted areas such as parks may favour the build up of internal parasite infections through contaminated feeding grounds and nutritional stresses.

Helminthes and protozoa are the two main kinds of internal parasites. Helminths can be divided into three categories; nematodes (roundworms), cestodes (tapeworms) and trematodes (flukes). Nematodes' life cycle is very simple and they are easily detectable in faecal matter, hence are the most commonly assessed helminthes with regard to animal health. Within the phylum Nematoda, members of the superfamily Strongyloidea (strongyles) have been observed in wild African and Asian elephants. This superfamily comprises the genera *Choniangium*, *Decrusia*, and *Equinurbia* as large strongyles and *Khalilia*, *Murshidia*, and *Quilonia* as small strongyles (Condy 1973; Gupta 1974; Chandrashekar 1992; Fowler 2006).

Adult nematodes live in the gastro intestinal tract of the host and produce eggs, which are expelled from the host with the faeces. First-stage larvae which hatch from eggs in deposited faeces moult two times and become third-stage larvae. The third stage larvae migrate from dung pats and soil onto moist vegetation. Infection occurs when third-stage larvae are consumed with fodder. The larvae complete their life cycle, becoming adults in the gastrointestinal tract of the host. Once the adult stage is reached, copulation occurs and the life cycle starts over (Fowler & Mikota 2006).

The classification of a parasite's importance is based on its disease-causing role such as prevalence, intensity of infection and pathogenicity. Gastrointestinal parasitic infection is responsible for most economic and production losses in livestock worldwide (Coop & Holmes 1996; Waller 2006). As eradication of parasites is not practical, the aim of control measures in livestock is to limit parasite populations to levels that are compatible with economic production (Brunsdon & Adam 1975).

Nematode diseases vary in severity from asymptomatic infection to rapidly fatal exsanguinations (Bowman & Georgi 2006). These parasites can affect host survival and reproduction directly through pathological

effects and indirectly by reducing host condition (Coop & Holmes 1996). The general effects of gastrointestinal nematode infection are partial or complete loss of appetite; interference with the production of digestive juices; damage to the lining of the alimentary tract so that materials such as proteins leak into the gut from the blood stream; diarrhoea which leads to dehydration; and possibly interference with digestion and the absorption of digested nutrients (Fox 2000).

The commonly used techniques of assessing gastrointestinal parasites are the 'faecal egg count' methods with the McMaster, flotation and sedimentation methods being the most widely used. Results gained from the McMaster method are quantitative whereas results from floatation and sedimentation methods are qualitative.

All the methods mentioned above have been developed for domestic animals. Compared to domestic animals, the faecal output of elephants is relatively high, which may decrease egg counts, necessitating the analysis of large volumes of sample. Elephant dung also contains undigested fragments that are very large, which interfere with laboratory procedures. Additional issues arise in the case of free ranging elephants due to logistics of collecting freshly deposited dung. Therefore there are a number of limitations in applying these methods to assess the faecal egg counts in elephants.

Here we describe a method, which is a combination of faecal culture of nematode eggs and harvesting through Berman technique to quantify the L₃ nematode larvae in free ranging elephants.

Materials and methods

Dung samples from free ranging elephants were collected in Galgamuwa in northwest Sri Lanka (Fig. 1). Laboratory analysis was done at the Animal Physiology Laboratory, Department of Animal Science, Faculty of Agriculture, University of Peradeniya, and at the Centre for Conservation and Research's field station in Galgamuwa.

Collection of faecal samples

Samples within 12 hours after defecation as judged from appearance (colour, odour, moistness, presence of insects) were collected.

Cleaning procedure for glasses and plastic ware

All glassware and plastic bottles used were thoroughly washed with tap water and detergent. They were then rinsed with tap water, followed by deionised water and air dried before use, to prevent any impact on larval growth.

Faecal culture of nematode larvae

12 g of fresh dung was measured using a digital kitchen scale and placed in a jar. The sample was broken up with a tongue depressor and moistened slightly with distilled water. The jar top was closed and placed on a shelf, away from direct sunlight, and incubated at room temperature for 7 days. The sample was stirred daily and additional water added if the dung appeared to be drying out.

After 7 days the sample was transferred to a 10 x 10 cm piece of double-layered cheesecloth and the edges of the cloth gathered and tied forming a bag. A short piece of rubber tubing was attached to the end of a glass funnel and the free end closed with a clamp. The funnel was placed on a rack. The tied cloth bag with the sample was



Figure 1. Collecting elephant dung samples.



Figure 2. Experimental setup.

suspended in the funnel by attaching to two applicator sticks resting on top of the funnel or to a wire strung above (Fig. 2). The funnel was filled with lukewarm water to cover the cloth bag and allowed to sit overnight for 24 hours. Then the cloth bag was removed and the liquid in the top of the funnel was siphoned off without disturbing the sediment at the bottom. A 10 ml quantity of 10% formalin was added to the sediment and mixed by shaking.

From the homogenized sediment, 0.5 ml was transferred to a 1 mm Sedgewick Rafter Counting Cell Slide (50 mm long x 20 mm wide and 1 mm deep) using a pipette and bulb, and examined under the compound microscope, using the 10X objective. L3 nematode larvae were identified based on their characteristic morphological appearance as a ‘tube within a tube’ referring to the alimentary canal which extends from the mouth on the anterior end, to the anus located near the tail. The chamber was scanned methodically and all larvae were counted. The process was

repeated till the entire sediment quantity was examined.

Sampling

In elephants, dung is deposited as a pile, consisting of a number of discrete boli. The following two sampling strategies were employed to assess sources of variance in egg distribution:

1. To determine variance due to non-uniform distribution of eggs within a bolus, ten samples were taken from each of three locations inside one bolus, making a total of 30 samples.
2. To determine variance due to non-uniform distribution of eggs among boli within a dung pile, five samples were taken from each of six boli in a single dung pile, making a total of 30 samples.

Statistical analysis

Data were analyzed using the computer program JMP 8. The non-parametric Wilcoxon / Kruskal-Wallis Tests were conducted to assess significance of observed differences.

Results

All samples analyzed yielded L3 larvae (Fig. 3). The mean number of larvae detected per sample was 297 ± 27 (range 374-220).



Figure 3. L3 larva under the microscope.

Table 1. Comparison of means for all pairs of boli using Tukey-Kramer HSD. Positive values show the pairs of means that are significantly different.

	Bolus 6	Bolus 4	Bolus 2	Bolus 5	Bolus 3	Bolus 1
Bolus 6	-39.6781	-19.0781	-7.0781	-4.4781	-0.6781	12.32193
Bolus 4	-19.0781	-39.6781	-27.6781	-25.0781	-21.2781	-8.27807
Bolus 2	-7.0781	-27.6781	-39.6781	-37.0781	-33.2781	-20.2781
Bolus 5	-4.4781	-25.0781	-37.0781	-39.6781	-35.8781	-22.8781
Bolus 3	-0.6781	-21.2781	-33.2781	-35.8781	-39.6781	-26.6781
Bolus 1	12.32193	-8.2781	-20.2781	-22.8781	-26.6781	-39.6781

Variance in parasite distribution inside a bolus

There was no significant difference (Wilcoxon match pair, $X^2=1.5177$, $d.f=2$, $P=0.4682$) in parasite numbers among the three sites within the same bolus (Fig. 4).

Variance in parasite distribution among boli within a dung pile

There was a significant difference in parasite number among two boli from the same dung pile (Fig. 5). The Tukey-Kramer HSD test revealed a significant difference between bolus 1 and bolus 6 ($P=0.0263$). No other inter-boli comparisons were significantly different (Table 1).

Discussion

Methodological aspects

Our results indicate that parasite distribution in elephant dung is fairly homogeneous but that some heterogeneity exists between boli, which is probably related to elephants' consumption of large volumes of food as mega-herbivores and mode of digestion as hind-gut fermentors.

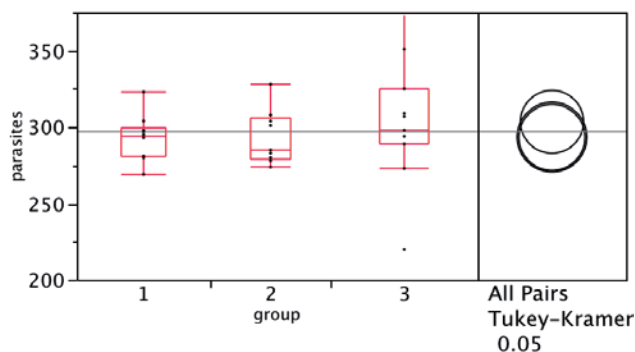


Figure 4. One-way analysis of parasites present in three locations in one bolus. Parasites = number of L3 larvae in 12 g of elephant dung.

There was no significant difference ($P>0.05$) in the parasite number in samples obtained from different locations within one bolus. Therefore, when sampling, it is sufficient to obtain a single sample from a bolus.

The parasite distribution was somewhat unequal among boli within one dung pile ($P<0.05$), with one of the 15 comparisons being significantly different from each other, indicating a significant difference between a single pair of boli. Therefore, when sampling, a composite sample should be taken to represent all boli in a single dung pile.

We recommend taking a single equal sized sample from within each bolus in a pile, homogenizing by breaking up the samples and mixing together, and taking a 12 g sub-sample for analysis.

Comparison with other methods

A number of limitations are encountered in estimating parasitic load using standard methods in free ranging animals. The McMaster, sedimentation and floatation methods for 'faecal egg count' estimation require samples to be

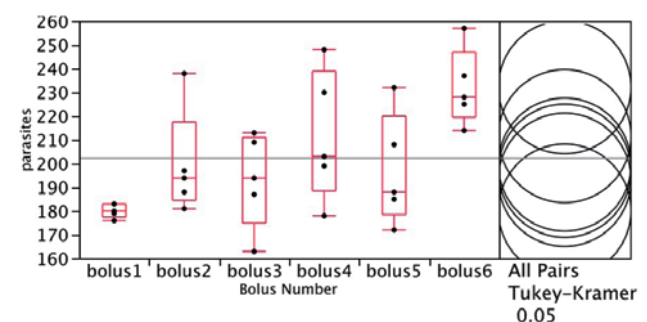


Figure 5. Number of parasites in 6 boli from one dung pile. Parasites = number of L3 larvae present in 12 g of elephant dung.

collected within 1 hour of defecation, as rapid embryonic development occurs in parasite eggs in the defecated faeces. Consequently these eggs will not float on to the surface of the salt solution used in the floatation method. Collection of samples within one hour of defecation in free ranging animals especially elephants is logistically difficult. This limitation is overcome in the method described here as the sample collection period was up to 12 hours. As the larval count was taken instead of the egg count in this method, the embryonic development process has no influence on the assessment.

In the McMaster method for 4 g of sample, 56 ml of floatation solution has to be added (Bowman & Georgi 2006). The volume of the floatation solution has to be increased in proportion to the weight of the sample. Therefore, to analyze a 12 g sample a large volume of floatation solution is required, making analysis tedious and cumbersome. This limitation in analyzing larger samples is overcome in the method presented.

Elephant dung contains a comparatively high percentage of very large fibrous and woody fragments. To analyze elephant dung using conventional techniques it has to be sieved and the large fragments removed, which makes analysis difficult and is likely to reduce the number of eggs recovered. The described technique is not affected by the size of fragments.

The main disadvantage of the described method is that it requires a 7 day incubation period, thus making the period of analysis long.

Conclusion

The described method provided results from all samples analyzed with comparative ease of processing and analysis. The technique provided fairly robust estimates of parasite numbers which lends itself to comparative analysis of parasite loads from different individuals and populations under free ranging conditions. We conclude that the developed technique can be used to successfully determine the intestinal nematode loads in elephants with comparative ease and reliability.

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