International Journal for Parasitology: Parasites and Wildlife 4 (2015) 43–48

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

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

Occurrence and seasonality of internal parasite infection in elephants, *Loxodonta africana*, in the Okavango Delta, Botswana

Lydia Baines [a], Eric R. Morgan [a,*], Mphoeng Ofthile [a], Kate Evans [a,b]

[a] School of Biological Sciences, University of Bristol, Tyndall Avenue, Bristol, BS8 1TQ, United Kingdom
[b] Elephants for Africa, P.O. Box HA148 HAK, Maun, Botswana

ARTICLE INFO

Article history:
Received 31 December 2012
Revised 4 January 2015
Accepted 6 January 2015

Keywords:
Nematode
Trematode
Coccidia
Elephant
Africa
Wetland
Transmission
Epidemiology

ABSTRACT

It is known from studies in a wide range of wild and domestic animals, including elephants, that parasites can affect growth, reproduction and health. A total of 458 faecal samples from wild elephants were analysed using a combination of flotation and sedimentation methods. Coccidian oocysts (prevalence 51%), and nematode (77%) and trematode (24%) eggs were found. Species were not identified, though trematode egg morphology was consistent with that of the intestinal fluke *Protofasciola robusta*. The following factors were found to have a significant effect on parasite infection: month, year, sex, age, and group size and composition. There was some evidence of peak transmission of coccidia and nematodes during the rainy season, confirmed for coccidia in a parallel study of seven sympatric domesticated elephants over a three month period. Nematode eggs were more common in larger groups and nematode egg counts were significantly higher in elephants living in maternal groups (mean 1116 eggs per gram, standard deviation, sd 685) than in all-male groups (529, sd 468). Fluke egg prevalence increased with increasing elephant age. Preservation of samples in formalin progressively decreased the probability of detecting all types of parasite over a storage time of 1–15 months. Possible reasons for associations between other factors and infection levels are discussed.

1. Introduction

Parasites can reduce body condition, reproductive success, and survival in their hosts (Irvine, 2006). Although parasite infections have been associated with mortality in the African elephant, *Loxodonta africana* (Vitovec et al., 1984; Obanda et al., 2011), research on the parasite fauna of this species is limited. More is known about parasites of Asian elephants (*Elephas maximus*), whose large captive population and significance to livelihoods underpin more detailed study (Lei et al., 2012). Apart from well recognised generalist taxa, most elephant-associated parasites so far described appear to be specific to either Asian or African elephants (Fowler and Mikota, 2006), suggesting that they have evolved to become host specific in the 7.6 million years since the African and Asian elephants diverged (Rohland et al., 2007). Due to the relatively limited amount of work that has been carried out on these parasites in African elephants, very little is known about their identity, occurrence, importance, life cycles and transmission dynamics.

Among African elephants, nematodes are frequently found (Kinsella et al., 2004; Fowler and Mikota, 2006; Thurber et al., 2011), with hookworms in particular reported to cause pathological lesions and haemorrhages in the bile ducts and liver, as well as the intestines (Obanda et al., 2011). The elephant-specific intestinal fluke *Protofasciola robusta*, likely to be an ancestral species within the Fasciolidae (Lotfy et al., 2008), has been associated with intestinal tissue damage, haemorrhage and death in free-ranging African elephants (Vitovec et al., 1984; Obanda et al., 2011). Coccidian infections, while apparently common, have not been widely associated with adverse clinical consequences (Fowler and Mikota, 2006).

This study sought to determine, by means of a coprological survey, the occurrence of and levels of infection with gastrointestinal parasites among African elephants in the Okavango Delta ecosystem, and to test for associations with potential drivers of transmission, including age, sex, group size and composition, season and year. Additionally, serial sampling of a small group of domesticated elephants at the study site was utilised to investigate the seasonality of transmission in this unusual and important part of the elephant's range.

2. Materials and methods

2.1. Study site

The study was conducted in the Ngamiland Wildlife Management Area 26 concession in the Okavango Delta, Botswana (19°25'N, 22°35'E).

* Corresponding author. University of Bristol, School of Biological Sciences, Tyndall Avenue, Bristol BS8 1TQ, United Kingdom. Tel.: +44 11739 41195.
E-mail address: eric.morgan@bristol.ac.uk (E.R. Morgan).

http://dx.doi.org/10.1016/j.ijppaw.2015.01.004

This is a private game concession of around 180,000 hectares, used for tourism, comprising riverine forest and grasslands, which flood seasonally along with the rest of the Delta. Rainfall is concentrated between November and February, and flood levels (from prior, upstream rains) rise from March to June, and then recede to September (Gumbricht et al., 2004). The study area has an estimated wild elephant population of 1350 (http://www.elephantdatabase.org/survey_aerial_total_count_strata/175, accessed 29th December 2014), as well as a small herd of domesticated African elephants used for transporting tourists on elephant-back safaris. Both populations have been subject to detailed behavioural studies in recent years (Evans and Harris, 2012; Evans et al., 2013), facilitating access to known groups and individuals for faecal sampling.

2.2. Faecal sampling

Fresh faecal samples were collected from individual free-living elephants during daylight hours (6 am–7 pm), between November 2008 and April 2012. Elephants were observed until they had defecated and had moved off to a safe distance. A sample was then taken, comprising separate aliquots from the surface and the interior of the dung bolus, to control for eggs having a heterogeneous distribution in the faeces. Only samples able to be collected within one hour of being dropped were taken. This was to avoid rapid parasite egg hatching or bolus drying, as well as disturbance and dispersion by insects such as dung beetles. Seven domesticated African elephants were kept at the study site. This group, known as the Abu herd, were used for elephant-back safaris, and enclosed at night but allowed to forage in the bush during daylight hours, as well as being walked regularly to water and on safari routes. This group therefore provided an opportunity to track temporal patterns in parasite load through longitudinal sampling, and hence reflect seasonal fluctuations in infection pressure to which wild elephants might also be exposed. Samples were collected from all seven members of the Abu herd over a shorter period (January to April, 2012), also during daylight hours. Samples were placed in plastic bags and stored in a cool box for transfer to the laboratory and processing on the day of collection.

The following information was collected for each sample: the date and time of collection, the age and sex of the elephant sampled, and the size and composition of its social group. Group composition was categorised as follows: Group 1 comprised either all females or females with males below the age of 15 years, while Group 2 comprised all males aged 15 years or more. These two categories represent the differential group living dynamics of wild African elephants. Female elephants remain in their matriarchal groups for life, while males are pushed out of these herds at the onset of puberty, and may remain solitary or form groups of their own (Evans and Harris, 2008, 2012). Elephants were assigned an age based on a number of visually observed variables, including body size, and tusk size and damage (Evans and Harris, 2008, 2012). The elephant population sampled has been the subject of long-term, on-going behavioural studies (Evans and Harris, 2012), and many of the observed elephants could be matched to a previously compiled identification database by observing ear markings, tusks and tail hair, and precise age consequently confirmed. This also minimised the risk of repeat-sampling of individuals.

For each sample collected between 12th November 2008 and 20th January 2012, three grams of faeces were weighed out, stored in a 15 ml storage pot and filled to the top with 10% formalin. These samples, hereafter referred to as formalin-preserved samples (FP-samples) were stored at ambient temperature and analysed between one and 15 months after collection. For samples collected between 21st January and 11th April 2012, hereafter referred to as unpreserved samples (UP-samples), three grams were also measured out, but were stored in a domestic refrigerator at around 4 °C, and analysed within 24 hours of collection.

2.3. Parasite enumeration

Nematode egg and coccidian oocyst density in faecal samples was estimated using a modified McMaster method (MAFF, 1986), with salt-sugar flotation solution (specific gravity 1.28) and a detection limit of 30 eggs per gram (epg). Briefly, 42 ml of water were added to each three gram sample, mixed thoroughly and then sieved. Two centrifuge tubes were filled with an aliquot of the sieved solution, and centrifuged for two minutes at 1500 rpm (400 *g*). The supernatant was then discarded and flotation solution added to the remaining sediment. The tubes were then inverted several times and a pipette was used to extract some of the mixed solution and place it in the chambers of a Fecpak slide (Fecpak Inc., New Zealand). This slide was used in preference to the standard McMaster slide because of the increased sensitivity, with one egg counted equating to 30 epg, compared with 50 epg using the standard modified McMaster method (Presland et al., 2005). The slides were left for two minutes to allow the eggs time to float to the surface before being examined under 10x objective (100 × total magnification) of a light transmission microscope. The prevalence of coccidian oocysts was recorded, and the number of nematode ova in each chamber was counted to estimate egg density.

Since some parasite ova, notably fluke eggs, could be too dense to float in salt-sugar solution, a sedimentation method (MAFF, 1986) was used to assess fluke prevalence. Faecal suspension was prepared as described in the flotation procedure above and topped up with water to 200 ml, mixed and poured into an inverse conical beaker. The beaker was left for three minutes to give fluke eggs time to sink. A pipette was then used to remove approximately 2 ml suspension from the very bottom of the beaker, and transfer it to the lid of a petri dish. After adding a drop of methylene blue stain, a graduated petri dish was then placed bottom-down on top of the lid to create an even layer of sediment, and the whole examined under 40x total magnification under a dissecting microscope. The number of fluke eggs seen was recorded. Early analysis of samples revealed that nematode eggs were frequently present in sediment fractions of FP-samples, while flotation tests on the same samples were negative for nematode eggs. Thereafter, nematode eggs were examined using both flotation and sedimentation methods. Sediment was examined in a petri-dish, as above, and the presence of fluke and nematode eggs recorded separately.

2.4. Statistical methods

Individual faecal samples were categorised by age, sex, month, season (wet, dry or flood), group size and group composition. Associations between these factors and the prevalence of coccidia and fluke, and of nematodes in FP-samples only, were investigated by binary logistic regression analysis, separately for each parasite type, in order to take account of potentially confounding interactions. All factors were included initially, and the least significant removed in turn until only significant predictors remained. The level of significance was set at $p = 0.05$. Logistic regression was not appropriate for nematode eggs in UP-samples, since observed prevalence was 100%. Instead, the effects of the same factors on nematode egg density were investigated by multiple linear regression analysis, following $\log_{10}(x + 1)$ transformation to stabilise the variance. Because of apparently inconsistent flotation of nematode eggs preserved in formalin, nematode egg density was analysed only for UP-samples, and prevalence of the three parasite categories analysed for UP- and FP-samples separately. Nematode egg counts from samples analysed only using flotation, before the limitations of this method were known (see section 2.3 above), were discarded from the analysis