CHAPTER 2

OBSERVATIONS ON OESOPHAGOSTOMUM BIFURCUM IN NON-HUMAN PRIMATES
A POTENTIAL RESERVOIR FOR HUMAN INFECTIONS IN GHANA


Submitted to the journal Parasitology
Abstract

In northern Togo and Ghana human infection with the parasitic nematode *Oesophagostomum bifurcum* (order Strongylida) is of major health importance. It could be assumed that non-human primates, the natural host of *O. bifurcum*, form a zoonotic reservoir for human oesophagostomiasis. In this study, faecal samples (*n* = 349) from the Olive baboon, the Mona monkey and the Black-and-white colobus monkey from two distinct geographical areas outside the human endemic area in Ghana were examined for the presence of *O. bifurcum* using both microscopy and species-specific PCR. The results showed a high percentage (75-99%) of the Olive baboon and Mona monkey samples to contain *O. bifurcum*. The majority of these test-positive samples contained a large number of larvae (> 100). No *O. bifurcum* was detected in the faeces of the Black-and-white colobus monkeys. Observation studies on the behaviour of the non-human primates, focusing on defecation, food consumption and physical contact with humans, indicated favourable conditions for zoonotic transmission. Given that no human infections with *O. bifurcum* have been reported from either study area, the present findings support the hypothesis that *O. bifurcum* from humans in the north of Ghana and *O. bifurcum* from the Olive baboon and/or the Mona monkey are biologically distinct.
Observations on *Oesophagostomum bifurcum* in non-human primates

**Introduction**

Human infection with *Oesophagostomum bifurcum* (Nematoda: Strongylida) is thought to be a rare zoonosis. However, it is endemic and of major human health importance in northern Togo and Ghana. There, at least a quarter of a million people are infected with this geo-helminth and in some villages the prevalence of infection is up to 70%. Infection with *O. bifurcum* causes significant disease due to the formation of granulomata and caseous nodules in the bowel wall of the large intestine, produced by encysted larvae.

Non-human primates are the natural host of *O. bifurcum*. Many different species of monkeys and baboons are known to be infected with *Oesophagostomum* species. Older reports make mention of serious pathological consequences from these infections. Therefore, it could be assumed that monkeys serve as a reservoir host in the area endemic for human oesophagostomiasis in northern Ghana and Togo. However, in this area the number of non-human primates has been greatly reduced and the remaining numbers are so small that they are now unlikely to play a major role in the local transmission of disease in humans. Elsewhere in Ghana there are still areas where large groups of non-human primates live in close association with humans. For instance, in Mole National Park (Northern Region, central Ghana) numerous Olive baboons (*Papio anubis*) live in and around the human settlements. Moreover, in the villages of Baobeng-Fiema (Brong Ahafo Region, central Ghana), Mona monkeys (*Cercopithecus mona*) and Black-and-white colobus monkeys (*Colobus vellerosus*) are constantly present. Both these places are tourist attractions and frequently visited by international travellers. However, to date no human infection with *O. bifurcum* have been reported from these areas.

While the prevalence and distribution of human oesophagostomiasis in northern Ghana have been studied extensively, it is unclear to what extent the non-human primates in this country harbour *O. bifurcum*. In the present study, faecal samples from Olive baboons from Mole National Park, and Mona monkeys and Black-and-white colobus monkeys from Baobeng-Fiema were examined for the presence of *O. bifurcum* by microscopy and species-specific PCR. Furthermore, observations were made on the behaviour of these non-human primates, focusing on defecation, food consumption and physical contact with the local people.
CHAPTER 2

Materials and Methods

Study areas and design
The present study was conducted at two distinct sites in Ghana, namely the village Yipala situated in Mole National Park (MNP) (Northern Region), and the two neighbouring villages Baobeng-Fiema in the Baobeng Fiema Monkey Sanctuary (BFMS) (Brong-Ahafo region) (Fig. 1). At these sites three species of non-human primates were studied, namely *Papio anubis* (Olive baboon) in MNP and *Cercopithecus mona* (Mona monkey) and *Colobus vellerosus* (Black-and-white colobus) in BFMS. Surveys in MNP were carried out from October 1999 till October 2000, with an intermission from February to April and in August. In BFMS the study period ranged from October 1999 till January 2000. The surveys were performed on a monthly base, with a time period of 7 to 15 consecutive days spent at each of the study sites. During these monthly periods direct observations and faecal sample collection of the non-human primates took place.

![Map of Ghana](image-url)

**Figure 1** Map of Ghana, indicating the *O. bifurcum* human endemic area and the two study sites Mole National Park (MNP) and Baobeng Fiema Monkey Sanctuary (BFMS).
Observations on *Oesophagostomum bifurcum* in non-human primates

Yipala is situated at the top of the escarpment in the South East corner of MNP (9°35' N and 2°26' W). The ~800 inhabitants of the village are families of the employees of MNP. In the village there is a primary school, a health post, training facilities for the National Wild Life Department and a small hotel for (international) visitors. MNP is Ghana's largest (~4,000 km²) and most popular game reserve, known for its elephant herds. It consists largely of open savannah woodland. Up to 100 species of mammals have been reported from this park, including five species of non-human primates: *Papio anubis; Erythrocebus patas; Ceropithecus aethiops; Colobus polykomos* and *Galago senegalensis*. The first of which, the Olive baboon 9 *Papio anubis*) is most frequently seen in and around Yipala.

BFMS is located in the Nkoranza district ~200 km south of MNP and 20 km north of the town of Nkoranza (7°43' N and 1°42' W). The area (~1.9 km²) is flat with a gentle slope and consists of plain forest, degraded forest, derived savannah and closed woodland. The sanctuary encompasses two villages, namely Baobeng (~1.000 inhabitants) and Fiema (~2.000 inhabitants), which are situated less than one km from each other. The community belongs to the Brong-Ahafo region and is sustained mainly by small scale farming activities. Both villages have a religious tradition of worshipping the two local species of monkeys (i.e., the Mona monkey and the Black-and-white colobus). In the 1970's the sanctuary was founded to guarantee the safeguard of the monkeys and to stimulate tourism. More information on the study areas, including detailed maps of Yipala and Baobeng-Fiema, is available on the website of Leiden University Medical Center (LUMC) (http://www.lumc.nl).

**Sample and data collection**

Non-human primates were observed daily and their behaviour was noted in a descriptive qualitative matter. These observational studies focused on places of sleeping, defecation, and food collection of the animals, as well as physical interaction with human activities.

Faecal samples were collected, mostly on a daily base, by picking fresh droppings from the ground at the sleeping places of the non-human primates. In FBMS, faecal samples were also collected from places where the monkeys scavenge for food. All sampling was done under the supervision of a professional game warden.

**Faecal sample examination**

Within 24 h of collection, a duplicate coproculture of each sample was carried out to identify third stage larvae (L3) of *O. bifurcum*. In brief, 2 grams of faeces were mixed with an equal amount of ‘vermiculite’ and placed on filter paper in a petridish under moist conditions for one week. Culture fluid was poured off and 100 µl of sediment was microscopically examined. L3s were identified morphologically using published keys and description. In addition, collected samples were microscopically examined for other parasitic infections, both helminths and protozoa, using direct smear, Kato-Katz thick smear technique and a formol-ether
sedimentation method according to Ridley. More details on these procedures and all related findings can be found on the website of LUMC.

Faecal samples were also subjected to specific PCR for the detection of *O. bifurcum*. For this purpose, samples were frozen within 24h after collection and subsequently transported to the Netherlands. DNA isolation and species-specific PCR’s were performed as described previously.45

**Results**

**Observations in Mole National Park**

In MNP, two groups (A and B) of non-human primates were observed in and around the village of Yipala. Group A consisted of ~15 Olive baboons, while group B was much larger and contained ~70 Olive baboons. During most of the day, group B was joined by a small number (n=2-5) of Patas monkeys. Although both groups of non-human primates moved separately through the whole study area, group A was mostly spotted around the primary school, while group B was regularly seen within the central village of Yipala and the nearby garbage disposal. Furthermore, both groups were frequently present around the hotel, searching for food and drinking water from the swimming pool. Both groups tried to steal food from the local people (Fig. 2a). Apart from what they got from these 'charges' (including yam, cassava, tomato ketchup, bread and bananas) the Olive baboons fed on grass, leaves, fruit and chicken or guinea fowl. During the night, the animals slept in trees in distinct areas of the park. No major fluctuations were detected in the daily pattern of activities of both groups during most of the study period, with the exception of the month July when the number of visits to the village area was significantly reduced.

Droppings of the baboons were mostly detected around their sleeping places, the garbage disposal, the primary school, the hotel and on the tracks between these places. In spite of the regular presence of non-human primates in the village, no faeces were observed in or around the houses of the local people. Other species of non-human primates in the area included *Ceropithecus aethiops*, *Colobus polykomos* and *Galago senegalensis*. However, these species were not seen in or around the village.

**Observations in Baobeng-Fiema**

In BFMS, ~400 to 500 Mona monkeys were present. They were divided over 17 to 19 different groups, each consisting of ~20-60 individuals. Although all groups visited the villages occasionally, most remained on the periphery. However, two groups of Mona monkeys were regularly present in the centre of the villages. In particular, one small group of around 20
Observations on *Oesophagostomum bifurcum* in non-human primates

animals, was spotted many times within the village of Baobeng, mostly early morning and/or late afternoon.

Figure 2  Olive baboons at MNP (a) and Mona monkeys at BFMS (b). Representative examples of sharing activities between the non-human primates and the local human population.
In the villages, the Mona monkeys stole food from the storehouses and from the courtyards, where people prepared and cooked their food. Also, they drank water from pots and pans that were used by the local people (Fig. 2b). Sometimes, the monkeys were fed by the inhabitants of the village and/or tourists. No particular defecation places were observed. Mona monkey droppings were seen at numerous locations; at the edge of the forest and the villages, between the houses of Baobeng-Fiema and at places where monkeys sat down to feed. In the forest outside the village, the Mona monkeys were mostly seen in the trees at low canopy height and sometimes on the ground. There the Mona monkeys fed on fruits and leaves.

The exact number of Black-and-white colobus monkeys in BFMS could not be determined, but is estimated to be ~400. These species of monkeys were mostly observed in the trees in the forest, though at a much higher canopy height compared to the Mona monkeys. They mainly fed on leaves. Except on one occasion, these species of monkeys were not seen within or around the villages. Also, no Black-and-white colobus droppings were found in the village, only in the forest area.

Parasitological findings
In total, 349 faecal samples of non-human primates were collected at MNP and FBMS and examined for *O. bifurcum* infection (Table 1). Microscopical examination after coproculture was performed on 279 of these samples. The results showed that 92% of the samples from Olive baboons and 75% of the samples from Mona monkeys contained *O. bifurcum* L3s. Of the positive-tested samples, 55% of the Olive baboon and 61% of the Mona monkey showed an extremely high intensity of infection (i.e., >100 L3 per 100 μl culture sediment). No *O. bifurcum* L3s were detected in the faecal samples of the Black-and-white colobus.

In MNP samples were collected over a time period of 13 months. The prevalence of infection with *O. bifurcum* in the samples from Olive baboons ranged from 71% (July) to 100% (several other months) without a clear seasonal pattern. Also in BFMS, no significant variation in prevalence of infection was detected between faecal samples collected in the four different months (data not shown).

From 125 faecal samples, DNA was extracted and subjected to species-specific PCR. The PCR results were in agreement with the coproculture results in that they showed high numbers of *O. bifurcum* positive samples of Olive baboons or Mona monkeys and no infections in the Black-and-white colobus monkeys (Table 1). For 55 samples both coproculture and PCR was performed. The *O. bifurcum* specific PCR was positive for 40 of the 42 coproculture-positive samples (95.2%). Also, six of the 13 samples in which no L3s of *O. bifurcum* were detected by microscopy were test-positive using the specific PCR.

In addition to *O. bifurcum*, several other species of parasites were detected in the samples used in this study. More details on these findings can be obtained from the LUMC website.
Observations on *Oesophagostomum bifurcum* in non-human primates

**Table 1** Outcome of 349 non-human primate faecal samples specifically diagnosed for *Oesophagostomum bifurcum* by coproculture (n = 279) and/or species-specific PCR (n = 125).

<table>
<thead>
<tr>
<th>Primate species</th>
<th>Coproculture</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Positives (%)</td>
</tr>
<tr>
<td>Olive baboon</td>
<td>173</td>
<td>159 (92%)</td>
</tr>
<tr>
<td>Mona monkey*</td>
<td>51</td>
<td>38 (75%)</td>
</tr>
<tr>
<td>Black &amp; white colobus</td>
<td>55</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*Mona monkeys showed a significant lower rate of *O. bifurcum* infection compared to Olive baboons (i.e., determined by coproculture (Chi-square = 11.2, P = 0.001) or species-specific PCR (Chi-square = 24.9, P < 0.001)).*

**Discussion**

The present study showed very high infection rates with *O. bifurcum* in Olive baboons and Mona monkeys in MNP and BFMS, respectively. Clearly, because examined stool samples could not be traced back to individual animals, no accurate prevalence could be determined within each population. Moreover, it cannot be excluded that more than a single faecal sample per individual animal has been collected and examined. Not only infection rates, but also infection intensities were high. When counting the number of L3s in the culture sediment, we found much higher numbers compared to reported studies in human infections. This indicates extremely heavy infections (i.e., worm burden) in the Olive baboons and Mona monkeys. However, an accurate comparison of intensity of infection between human and the non-human primates is not possible because exact quantities of stools excreted are unknown. Nonetheless, the findings of the present study make it clear that there is a highly effective transmission in the Olive baboons and Mona monkeys. On the other hand, the aboreal (i.e., living in trees) Black-and-white colobus monkeys appear not to be at risk of this geo-helminth infection.

The fact that the microscopical findings were confirmed by species-specific PCR rules out the possibility that the observed L3s represented other morphologically similar species of *Oesophagostomum*, such as *O. colombianum*, *O. dentatum* or *O. quadrispinulatum*. These could be present in the environment (i.e., water, soil and dust), or on vegetation (i.e., grass or plants) in MNP and BFMS, due to transmission by other local animals like goats or warthogs.
To date, no cases of human infection with *O. bifurcum* have been reported from the study area. Moreover, around 90% of all inhabitants of MNP (n = ~700) and a selection of individuals from BFMS (n = ~100) have been extensively examined so far by stool culture and/or PCR for the presence of nematodes. All collected stool samples were shown to be test-negative for *O. bifurcum*, while *N. americanus* infections were commonly present. Regular treatments with anthelminthics like albendazole were not known to occur previously to or during the period of examination. (Polderman et al, unpublished)

The complete absence of *Oesophagostomum* in humans in MNP and BFMS is remarkable for three reasons. Firstly, the conditions for transmission seem to be highly favourable: the infection rates and apparent intensities of infection in monkeys and baboons are high, with no major seasonal fluctuations. This indicates that the free-living, potentially infective, larval stages successfully develop throughout the year under the prevailing environmental and ecological conditions. Secondly: the observations on the animal behaviour, described in the present paper, indicated a close interaction between human and non-human primates by sharing the same habitat. The intensity of contacts is difficult to quantify. Still, the frequency of finding faecal samples of non-human primates in, or in the close vicinity of the human compounds and the mere co-existence of some 3000 people and over 400 Mona monkeys, sharing a surface no more than about 1.9 km$^2$ are impressive indications for the closeness of the monkey-human interaction. Thirdly, humans have shown to be able to be perfectly suitable hosts for *O. bifurcum*, as demonstrated by the high prevalence of human infections, just a few hundred kilometres to the north of MNP and BFMS. Admittedly, in the human endemic region too, details of the route of transmission remain unclear. For instance, while it is believed that transmission of *O. bifurcum* L3s to humans is oral, it cannot be excluded that it can also occur percutaneously. Nevertheless, if transmission to humans can efficiently take place in northern Ghana, it is a challenge to understand why it should not be able to occur in NP and BFMS, where the parasite is abundantly present and human exposure seems to take place.