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New Evidence for Leaf Swallowing and *Oesophagostomum* Infection in Bonobos (*Pan paniscus*)

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We collected data on parasitic prevalence and leaf-swallowing behavior of bonobos (Pan paniscus) between August 1998 and April 1999 at the Iyema research site, Lomako Forest, Democratic Republic of Congo. We report the first detailed observations of leaf-swallowing among bonobos and the first record of the behavior at Iyema-Lomako. Bonobo leaf-swallowing closely fits the description of the behavior among chimpanzees. Bonobos ingested leaves of Manniophyton fulvum, as occurs in two chimpanzee populations in Central and Western Africa and among bonobos at Wamba, about 200 km from Iyema-Lomako. All leaf-swallowing occured in the rainy season. In conformity with patterns among Mahale chimpanzees, the prevalence of Oesophagostomum sp. infection in bonobos increased after the onset of the rainy season.

KEY WORDS: Pan paniscus; Oesophagostomum stephanostomum; leaf swallowing.

INTRODUCTION

Since Janzen (1978) suggested the possible use of plant secondary compounds as medicine by animals, the evidence for self-medication in

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African great apes has grown (Huffman, 1997; Huffman *et al.*, 1993, 1996; Huffman and Seifu, 1989; Huffman and Wrangham, 1994; Wrangham, 1995; Wrangham and Goodall, 1989; Wrangham and Nishida, 1983). Selfmedication in the great apes is best documented for the well habituated chimpanzee communities at Mahale, Gombe and Kibale. The two main patterns distinguished are bitter pith chewing and whole leaf-swallowing, which are antiparasitic. The benefits of bitter pith chewing is pharmacologically based on the activity of steroid glucosides (Jisaka *et al.*, 1993; Ohigashi *et al.*, 1994).

The hypothesized nematocidal action of leaf-swallowing (Page *et al.*, 1992; Rodriguez *et al.*, 1985; Rodriguez and Wrangham, 1993) has not been supported by evidence (Huffman *et al.*, 1996; Page *et al.*, 1997). Instead the mode of action of leaf-swallowing is physical, based on the rough hispid surface of the leaves (Huffman *et al.*, 1997, Huffman and Caton, 2001). Leaf-swallowing facilitates expulsion of tapeworm fragments by Kibale and Budongo chimpanzees (Huffman, in prep.; Wrangham, 1995) and of the adult nematodes (*Oesophagostomum stephanostomum*) by Mahale chimpanzees (Huffman *et al.*, 1996). An important fact demonstrating the role of the behavior in parasite control is synchrony of leaf-swallowing and a rise in *Oesophagostomum* infections (Huffman *et al.*, 1990, 1997; Kawabata and Nishida, 1991) with onset of the rainy season. Huffman *et al.* (1997) found that the increase in infection became notable approximately 3–6 weeks after the onset of the rainy season.

Knowledge of intestinal parasites in wild bonobos (*Pan paniscus*) is limited to two surveys (Dupain *et al.*, 1999; Hasegawa *et al.*, 1983). Both studies demonstrated the presence of *Oesophagostomum sp.* infections. In addition, Wamba bonobos swallow whole leaves of *Manniophyton fulvum* (Huffman, 1997).

In 1998, we started a study on the prevalence of intestinal parasites in sympatric bonobos and humans in conjunction with the use of medicinal plants in the Lomako Forest, Equateur Province, Democratic Republic of Congo (DRC). We conducted the study at the Iyema site maintained by the Centre for Research and Conservation of the Royal Zoological Society of Antwerp, Belgium. During the first phase of this study, we obtained both direct and indirect evidence of bonobos swallowing leaves of *Manniophyton fulvum*.

The preliminary evidence allows us to consider the possible role of leaf-swallowing as a form of self-medication in bonobos. We aim first to describe the leaf-swallowing evidence and secondly to present the results of a survey on parasitic prevalence and leaf-remains in bonobo dung. We will discuss the results in light of the control of parasitic infection hypothesis first proposed by Huffman *et al.* (1996, 1997) for chimpanzees. Conditions

supporting the hypothesis would be an increase in the prevalence of *Oesophagostomum* infections at the onset of the rainy season and evidence for increased leaf swallowing behavior then.

MATERIALS AND METHODS

Study Site

The Iyema study site, is in the Lomako Forest, Equateur Province, Democratic Republic of Congo (Dupain *et al.*, 2000). The study group is partly habituated. We individually recognized 12 of *ca.* 50 members of the community. Nell and Dupain collected data between August 1998 and April 1999. Data collection was discontinuous because of local warfare, and two of the researchers were held captive by soldiers. We observed the bonobos for 10 h in August and 27 h in October 1998.

Seasonality

Climatological data were only partly available for October and November 1998. Based on our records of weather conditions during our stay in Mbandaka and Basankusu (150 and 300 km respectively west of Iyema-Lomako) in September, and the information from local collaborators about rain conditions at the study site in September, the onset of the heavy rainy season—first month with >200 mm rainfall—occurred in October (4–30 October: 202 mm). The dry season (<100 mm/month) started in January.

Dung Collection and Analysis

We collected all dung samples freshly in the morning under bonobo night nests. Accordingly, on any given day we could measure the random group estimates of parasitic prevalence as individuals were only sampled once. For parasitological analysis, we used the 86 faecal samples collected in October–November 1998, rainy months. They were immediately diluted 1:3 in formalin and transported for analysis to the Department of Parasitology, Fac. Pharmacy, Universidad Complutence de Madrid, Spain. Ponce and Garcia performed the analysis (<u>Ritchie, 1948</u>). We observed the samples under 16–64x magnification after resuspension in PSB, and under light/phase contrast microscopy at 100–400x magnification. We examined the samples for intestinal parasites. From the results, we assessed the increase in the prevalence of *Oesophagostomum*, *Troglodytella* and *Strongyloides* infection after the onset of the rainy season in two ways. Via regression analysis we tested for a correlation between time and rate of infection. We used the two-tailed Fisher's exact test to test for a significant increase of parasitic prevalence between October and November.

RESULTS

Leaf-Swallowing

Direct Evidence

We observed leaf-swallowing on 20 October:

- 0750 h We relocate a party of 8 individuals *ca*. 60 m from the previous night nest site. In the previous hour, we had observed foraging on *Gambeya lacourtiana* and foraging by an adult female (Tsjoep) with infant in a liana (*Manniophyton fulvum*). She sits 15 m up in a tree. An adult male (Mobali) sits in the same tree at 10 m *ca*. 5 m from Tsjoep.
- 0755 h Tsjoep is sitting in the same place. Mobali climbs into the liana to a height of approximately 13 m. He takes two leaves, moves up the branch 2 more m and sits down. With his right hand, he slowly puts it into his mouth and swallows it without any apparent sign of chewing. Meanwhile he holds the second leaf in his left hand.
- 0800 h Mobali puts the second leaf into his mouth in the same slow way and swallows it without chewing.
- 0801 Mobali starts to travel; Tsjoep and the others follow him.

Dupain climbed into the liana and collected leaves. L. Pauwels (National Botanical Garden of Belgium) confirmed them to be *Manniophyton fulvum* (Euphorbiaceae).

Indirect Evidence

We analysed a total of 131 faecal samples to examine for the presence of hispid leaf-remains; 98 during the rainy and 33 during the dry season (Table I). We found unchewed leaf remains of *Manniophyton fulvum*, which varied in size (>10 cm², <5 cm²) in two different samples in the rainy season. We also recorded the remains of two *Cola spp.* in 9 dung samples, 8 of which were from the rainy season. None of them remains was >5 cm².

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	Rainy season $(n = 98)$	Dry season ($n = 33$)
Manniophyton fulvum	2	0
Cola sp.	6	0
Cola bruneeli	2	1

Table I. Presence of unchewed leaf portions in fecal samples of Lomako bonobos

Parasitic Prevalence and Seasonality

We found a significant increase in the prevalence of *Oesophagosto*mum infection during the first weeks of the rainy season. There is a significant positive correlation between time and rate of infection (r=0.731; F1,12=13.8, p<0,05) (Fig. 1), but not for *Troglodytella* (r=-0,13, F1,12=0,2, p>0,5) or *Strongyloides* (r=0,288, F1,12=1,1, p>0,5). Furthermore, only *Oesophagostomum* infection rates differ significantly between October and November (Fisher's exact test, two-tailed p value=0.05), versus infection rates of *Troglodytella* and *Strongyloides* (Fisher's exact test, two-tailed p value>0.1,>0.1 resp.) (Table II).

DISCUSSION

This is the first detailed published record on leaf-swallowing behavior by bonobos and the first record of this behavior at Iyema-Lomako. We confirmed leaf-swallowing by direct observation and indirectly by dung sample analysis.

An important element of the indirect evidence demonstrating the role of leaf-swallowing in parasitic control by chimpanzees is the synchrony of *Oesophagostomum* (tapeworm) infection and the occurence of leaf-swallowing (Huffman *et al.*, 1996, 1997; Wrangham, 1995). Wrangham (1995) focused on an outbreak of *Bertiella studeri* proglottid expulsion and Huffman *et al.* (1996) focused on the seasonal increase of *Oesophagostomum stephanostomum* infection and expulsion of adult worms with leaves. The significant increase in infection rates of *Oesophagostomum* in chimpanzees during the rainy season was first described by Huffman *et al.* (1990). As *Oesophagostomum* is listed as a common parasite for bonobos (Dupain *et al.*, 1999; Hasegawa *et al.*, 1983), and we observed them swallowing of *Manniophyton fulvum* leaves, we wanted to compare their incidences at Iyema-Lomako for similarity with observations in Mahale chimpanzees. Due to the life cycle of *Oesophagostomum*, we expected an increase of infection by during the rainy season (Huffman *et al.*, 1997).

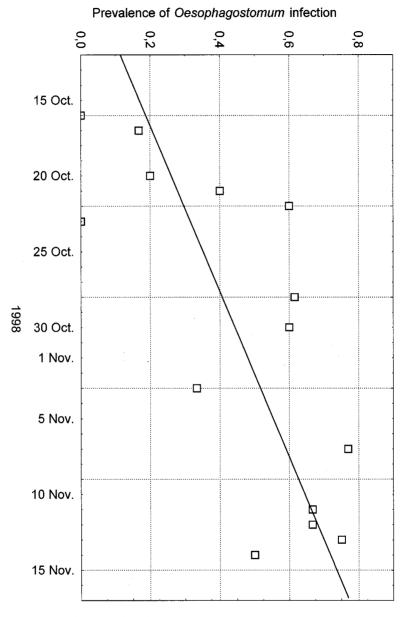


Fig. 1. Regression analysis of time vs. prevalence of occurence of *Oesophagostomum* infection in Lomako bonobo dung samples collected at the onset of the rainy season (R = 0.73, F = 13.8, p < 0.005).

Month (No. of samples)	Oesophagostomum*	Troglodytella	Strongyloides
October (48) November (38)	19 (40%) 24 (63%)	39 (81%) 25 (71%)	16 (33%) 15 (39%)
Total (86)	43 (50%)	64 (74%)	31 (36%)

 Table II. Prevalence of Oesophagostomum, Troglodytella and Strongyloides infection in dung samples of Lomako bonobos collected in October–November 1998

 $p^* = 0.05$, Fisher exact test: two-tailed.

The rainy season started at the end of September/beginning of October. Heavy rains continued until December. The dry season started in January. We collected samples for parasitological analysis in the rainy season. Infection by *Oesophagostomum* has a minimum prepatent period of 3 weeks (Anderson, 1992). Therefore, we expected a gradual measurable increase in the prevalence of parasitic infection from the second half of October on as the infective larvae mature to adulthood and they begin to lay eggs, with an increasingly higher rate of infection in the second month after the onset of the rainy season. We analyzed simultaneously for the prevalence of infection of two other chimpanzee intestinal parasites, *Strongyloides* and *Troglodytella*, known for their lack of seasonality (Ashford *et al.*, 2000; Huffman *et al.*, 1997; Kawabata and Nishida, 1991) as control measures for sample bias.

We confirmed a significant increase in the prevalence of *Oesophagos-tomum* infection in Iyema-Lomako bonobos via a significant correlation between time and the rate of infection during the study period and by the significantly higher prevalence of infection in November versus October. The increase is further confirmed by the lack of correlation with time and by the even distribution over the rainy season of *Troglodytella* and *Strongy-loides* infection. As expected, evidence for leaf-swallowing was predominant in the rainy season.

The observation of bonobo swallowing leaves of *Manniophyton fulvum* closely resembles the description of leaf-swallowing in *Pan troglodytes* (Huffman and Caton, 2001; Wrangham and Nishida, 1983). The leaves were selected in the early morning, (0755 h) within about 1–2 h after leaving the night nest, and they were swallowed singly and slowly. The presence of leaves of *Manniophyton fulvum* in dung samples on two separate occasions confirms the probably habitual nature of leaf-swallowing. We also found remains of other hispid leaves (*Cola bruneeli* and *Cola sp.*) in dung samples and found remains from feeding on leaves of *Cola sp.* on one occasion early in the morning. Idani *et al.* (1994) observed bonobos eating leaves of *Cola bruneeli* and *Cola chlamydantha* but provided no information on whether they were swallowed or chewed. Given the rough hispid characteristic of the leaves, *Cola spp.* are possible candidates for leaf-swallowing by bonobos. Leaf-swallowing and regular chewing are not mutually exclusive. Mahale and Bossou (Guinea) chimpanzees both chew and swallow the rough leaves of *Ficus exasperata* at different times of the day, demonstrating a dual but strictly seperated nutritional versus self-medicative use of the same species of leaf.

There is a tendency for apes in neighboring regions to select the same plant species for leaf-swallowing. For example, in eastern Africa, Mahale, Kibale and Gombe chimpanzees swallow leaves of *Aneilema aequinoctiale* (*Commelinaceae*), and Mahale and Gombe chimpanzees swallow leaves of *Hibiscus aponeurus* (*Malvaceae*) (Huffman, 1997). Our observations of bonobos swallowing the leaves of *Manniophyton fulvum* further support the reports from three other sites in Central and West Africa where apes also swallow leaves of *Manniophyton fulvum*. (Boesch, 1995; Huffman and Wrangham, 1994).

Leaf-swallowing by Lomako bonobos closely resembles leaf-swallowing in Kibale, Mahale and Gombe chimpanzees. Furthermore, our data support the increase in prevalence of *Oesophagostomum* infections in bonobos during the rainy season, as reported for the Mahale chimpanzees. These common elements in the behavior of Lomako bonobos and leaf-swallowing by apes elsewhere provide strong support for the hypothesis that leafswallowing might be wide spread in bonobos.

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