Diagnosis, transmission and immunology of human Oesophagostomum bifurcum and hookworm infections in Togo

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Chapter 6

The patterns of infection and re-infection with *Oesophagostomum bifurcum* and hookworm following treatment in northern Togo

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SUMMARY

Until recently human infections with *Oesophagostomum bifurcum* were considered as rare zoonotic infections. In northern Togo however, approximately 30% of the population is infected with *O. bifurcum*, while some 70% is infected with hookworm. In order to understand the mechanism of transmission, the seasonal changes in larval counts are carefully monitored in groups of subjects treated before and after the rains.

Albendazole, the drug of choice for the treatment of individuals with mixed infections, was shown to have good cure rates for *Oesophagostomum* but very modest ones for hookworm, in this region. In this study treatment of population groups at different seasons of the year showed that reinfection was confined to the rainy season and that larval counts varied considerably from one rainy season to the other. The data are consistent with the idea that development of some of the larvae may be arrested for considerable times. Treatment of the whole population before the rains was followed rapidly by a resumption of larval output. Following treatment after the rains, the larval counts remained low until the following rainy season. This distinct pattern of transmission of the parasite will aid informed decisions on the optimal treatment scheme to apply.

INTRODUCTION

Although generally considered as a common nematode parasite of monkeys, *Oesophagostomum bifurcum* is highly endemic among the human population of northern Togo, and causes significant morbidity. In addition, more than 70% of the population of northern Togo is infected with hookworm (*Necator americanus*) (Polderman, *et al*, 1991).

Our understanding of the life cycle of *O. bifurcum* is based on what is known from *Oesophagostomum* species of veterinary importance. After oral ingestion the infective third-stage larvae penetrate the intestinal wall for the first part of their development. Immature worms then re-enter the intestinal lumen to mature and start egg production (Dash, 1981).

In many nematode infections, including those of *Oesophagostomum ssp.* in ruminants and pigs, some of the larvae may enter a stage of arrested larval development (ALD). They remain encapsulated within the intestinal wall until development is resumed. ALD can be seen as a strategy of the parasite to overcome the harsh conditions of an adverse immune environment, an overcrowded habitat, or environmental conditions which are hostile to the free-living stages of next generation (Armour & Duncan, 1987). It is likely that this mechanism plays a role in human *Oesophagostomum* infections as well. If it does, it may have an important impact on the epidemiology and it may interfere with the outcome of treatment.

Earlier observations suggested that transmission of *O. bifurcum* in humans is mainly confined to the rainy season (Krepel, *et al.*, 1995a). Such observations are plausible considering the combination of high temperatures and low precipitation and humidity during the long dry season. Laboratory studies, however, demonstrated that the L3-larvae of *Oesophagostomum bifurcum* are able to survive long periods of desiccation and can be revitalized following rehydration (Polderman & Blotkamp, 1995, Pit *et al*, in press). It was even shown that desiccated and subsequently rehydrated larvae can cause patent infections in monkeys (Eberhard *et al*, submitted). Transmission in the middle of the dry season with desiccated larvae can therefore not be ruled out.

Clearly, transmission itself cannot be easily measured. Instead it is the effect of transmission, i.e. the eggs excreted with the host's stools, that is normally used as an indirect parameter for transmission. Fluctuations in egg counts, and in the numbers of L-3 larvae cultured, over the seasons may have many different causes: day-to-day variation in egg-output, seasonal variations in stool consistency, in nutritional status of the host, in egg production by the female worm, (Scott, 1938; Pit *et al.*, 1999). It may also be the result of arrested larval development. The use of egg counts to understand transmission and to recognize ALD is, therefore, likely to result in a variety of erroneous conclusions.

Albendazole kills the adult *O. bifurcum* and hookworms in the intestinal lumen (Krepel *et al.*, 1993), and it probably also kills the tissue dwelling stages (Storey *et al.,* submitted). The potential of this drug for use in mass treatment campaigns with the objective of transmission reduction of *O. bifurcum* still has to be determined.

The main objective of the present study was to obtain a better understanding of the transmission of *Oesophagostomum*, in particular of those mechanisms that enable the parasite to overcome the long dry season. We therefore carefully monitored seasonal changes in larval counts in groups of subjects treated before or after the rains and compared with untreated controls. As a practical consequence, it is attempted to decide on the optimal time of the year to apply population-based treatment.

MATERIALS AND METHODS

Study area

The fieldwork was carried out in a rural area 25 km west of Dapaong (northern Togo, West Africa). The estimated 4000 inhabitants of the area live in small gatherings of clay huts called "soukoula's". The area comprises the communities of Lotogou and Tampialime. The inhabitants grow millet, maize and cotton. Pigs, goats, sheep, and cattle are raised close to the house. Water from a borehole is used for drinking, cooking, and washing. In the rainy season some people may also fetch water from small ponds. There are a few latrines, but most people squat in the open fields. The rainy season lasts from April to October, with an annual rainfall of 855 mm. The rest of the year is dry and hot with temperatures up to 40 ^{0}C. In November, the cool Harmattan wind sets in and temperatures can drop to 15 ^{0}C at night.

Study population

Households living in close proximity to each other made up the study group in both villages, and volunteered to participate with their whole family, after having been duly informed of the purpose of the study, in their own language. Informed consent was obtained from four hundred individuals, between 1 and 70 years. The demographic characteristics of the area are homogeneous in ethnicity and socio-economically. The final analysis was confined to a total of 197 individuals who participated in at least 15 out of 19 surveys, representing a good compliance of 48%. No significant differences were observed between the complying and non-complying group, concerning age and sex distribution, or prevalence and intensity of infection at the onset of the study.

The participants were randomly assigned to one of the four treatment groups: Group 1 (n= 50) acted as a control group and no treatment was given until the end of the study. Group 2 (n= 61) was treated in May 1995 at the onset of the rainy season, immediately after the first survey; Group 3 (n= 40) was treated in September 1995, towards the end of the rainy season; and Group 4 (n= 46) was treated in December 1995 in the middle of the dry season. No significant differences were observed in age or sex distribution between the different treatment groups. Following local protocols, Albendazole was given in single oral doses of 200 mg for a body weight of less than 40 kg, 400 mg for a 40 to 60 kg body weight and 600 mg for individuals over 60 kg. Compliance was ensured by supervised tablet swallowing. The entire population, including the control group, was treated at the end of the study.

Stool samples and parasite-specific diagnosis

Stool samples were taken every month from May 1995 until November 1996. Individually labeled plastic containers were distributed to the participants and collected the next day. Individuals who did not donate stools on the collection day were allowed an extra day to do so. The eggs of *O. bifurcum* are morphologically identical to those of hookworm. Only the third-stage larvae of both parasites, obtained by coproculture, show distinguishable morphological features (Blotkamp *et al*, 1993). Therefore a duplicate coproculture was made of each collected stool sample. Briefly, a quantity of 3 grams of faeces was mixed with an equal amount of vermiculite, placed on moist filterpaper in two Petri dishes and incubated at room temperature (25-35 °C). After seven days the water was poured off into a conical tube, the Petri dish was rinsed and the water added to the conical tube. After sedimentation for at least 2 hours, 100μl of the sediment were examined at low magnification (4x10) for the presence of larvae. The *O. bifurcum* and *N. americanus* larvae were individually identified and counted. The total number of larvae of each species in the duplicate coprocultures was used to indicate the intensity of infection. When one of the duplicate cultures was spoiled, the number of larvae found in one coproculture was multiplied by 2 (Krepel *et al.*, 1993; Little, 1981).

Data analysis

Prevalences of infection are given as the percentage of parasitologically positive individuals in the total study population. The intensities of infection are expressed as fractions of the subjects with "heavy infections" (i.e. more than 32 larvae per 3 g coproculture) (Krepel *et al.*, 1995b). Larval counts were highly skewed, even after logarithmic transformation, therefore median, 25^{th} and 75^{th} percentile values were used. Individuals were grouped into five age groups reflecting young childeren (0-4 years), childhood (5-9), adolescence (10-19), early adulthood (20-39) and late adulthood (over 40). Age-group

Tabel 1: Characterization and parasitological data of the study population.
[a]On the first survey (n= 197); [b]cumulative prevalence of infection in the control group after 19 surveys (n= 50).

Male/female:	100/97
Median age (range):	11 (1-70)
O. bifurcum:	
% infected[a]	68%
Median larval count/3g faeces[a]	5
(25th and 75th percentile)[a]	(0-21)
cumulative prevalence[b]	86%
hookworm:	
% infected with[a]	82%
Median larval count/3g faeces[a]	16
(25th and 75th percentile)[a]	(2-59)
cumulative prevalence[b]	94%

differences in prevalences were analyzed by Chi-square test, and intensity of infection by Mann-Whitney and Kruskal-Wallis non-parametric tests on untransformed data. Arbitrary cure rates were calculated as the percentage of infected individuals becoming parasitologically negative one month after treatment.

RESULTS

The characteristics of the study population and the prevalences of infection are summarized in table 1. The cumulative prevalence of infection with *O. bifurcum* as well as with hookworm was very high. The frequency distributions of *O. bifurcum* and hookworm larvae were highly aggregated, i.e. a small proportion of individuals harbored a large proportion of parasites; with 50% of the *O. bifurcum* and hookworm larvae being produced by 7% and 10% of the individuals, respectively.

There were significant differences in prevalence of infection and larval counts of both parasites between the different age groups ($P < 0.015$, Fig. 1). The highest prevalence of infection was found in adolescents (10-19 years).

Fig. 2 (a, b) shows the prevalence of infection with *O. bifurcum* and

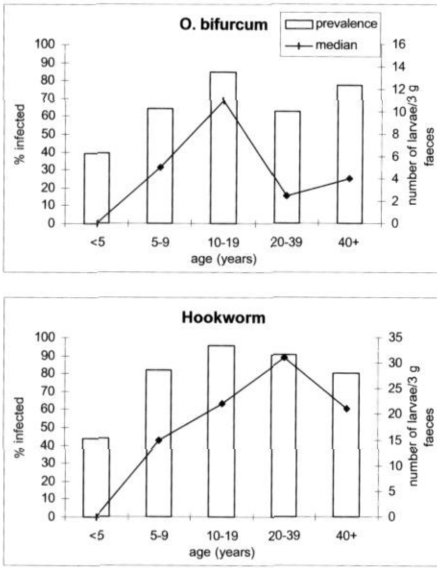

Fig. 1: Age-related prevalence and intensity of infection with O. bifurcum and hookworm.

hookworm during 19 consecutive months without chemotherapeutic intervention (group 1). During the first rainy season the prevalence of infection remained relatively constant for both parasites, but the intensity of infection increased. In September more than 30% of the study group was heavily infected with *O. bifurcum* and in more than 55% the larval counts for hookworm were high.

During the dry season the excretion of eggs diminished, such that not only the intensity of infection but also the prevalence decreased. For both parasites the lowest prevalence of infection was in April, just before the start of the rainy season. Without treatment, at the onset of the next rains, both prevalence and intensity of infection increased again, following a similar pattern to that observed during the previous rainy season, but the intensities of infection tended to be somewhat lower.

Figure 3 shows the effect of Albendazole on prevalence and intensity of reinfections, when treatment was given at different time points during

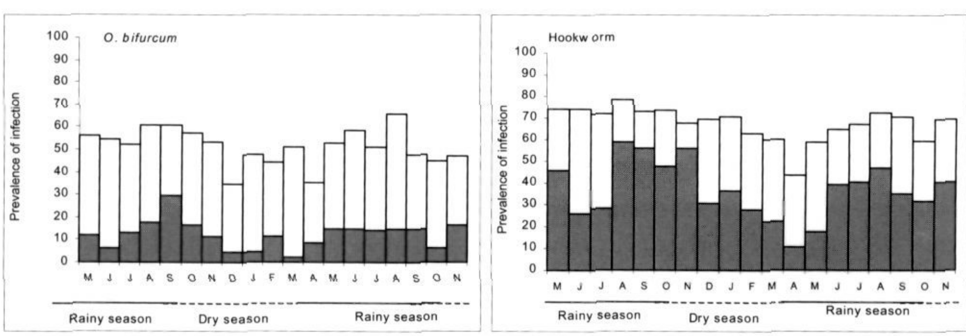

Fig. 2: Prevalence and intensity of infection with O. bifurcum (a) and hookworm (b), in an untreated population in northern Togo, during 19 consecutive month

the year. The average arbitrary cure rate with Albendazole was 87% for *O. bifurcum* infections and 57% for hookworm infections. Group 2 was treated after the first survey in May, at the start of the rainy season (Fig. 3a). Ninety three percent (93%) of the *O. bifurcum* infections were cured. During the next months of rains, prevalence and intensity of infection with *O. bifurcum* increased, and 5 months post-treatment (p.t.), in October, they were similar to pre-treatment levels. From then on the monthly prevalence and intensity of infection of group 2 were always similar to those of group 1. Group 3 was treated in September at the end of the rainy season (Fig. 3b). A cure rate of 72% was achieved, after which prevalence and intensity of infection with *O. bifurcum* remained at the same low level during the whole dry season, not increasing again until the beginning of the rains. Even then, infection prevalence and intensity remained lower compared with group 1 and did not return to pretreatment levels. Group 4 was treated in December in the middle of the dry season (Fig. 3c). The cure rate was 88%. Again, the majority of the people remained free of *O. bifurcum* infection until the start of the rains in May. Although intensity of infection was high in some people during the following rainy season, prevalence

of infection did not increase to the same levels found before treatment. Reinfection with hookworm showed a pattern similar to *O. bifurcum,* with reinfection confined to the rains. The group treated in May (cure rate 66%) became quickly reinfected, and within 5 months prevalence and intensity of infection equaled the values from before treatment (Fig. 3d). The group treated in September showed a poor cure rate of 52%, but prevalence and intensity of infection did not increase until the middle of the following rainy season (Fig. 3e). In the group treated in December (cure rate 49%), prevalence and intensity of infection increased only slightly with the start of the rains (Fig. 3f).

DISCUSSION

The observed distribution of worms per person was highly aggregated; i.e. a majority of worms were harbored by a minority of the population. Aggregation to hookworm infection are well known and have been reported previously (Schad & Anderson, 1985; Haswell-Elkins *et al* 1987,1988; Bradley & Chandiwana, 1990 and Quinnel *et al,* 1993) and are thought to be associated with behavioral patterns (i.e. choice of defaecation site, social factors), intrinsic factors to the genetic background (e.g. immunoresponsiveness) as well as the nutritional status of the host (Anderson & May, 1982; Anderson & Medley, 1985; Haswell-Elkins *et al*, 1987). The practical relevance of this observation in planning control is small since reliable determination of the intensity of infections is cumbersome and time consuming.

Therapeutic intervention with Al-

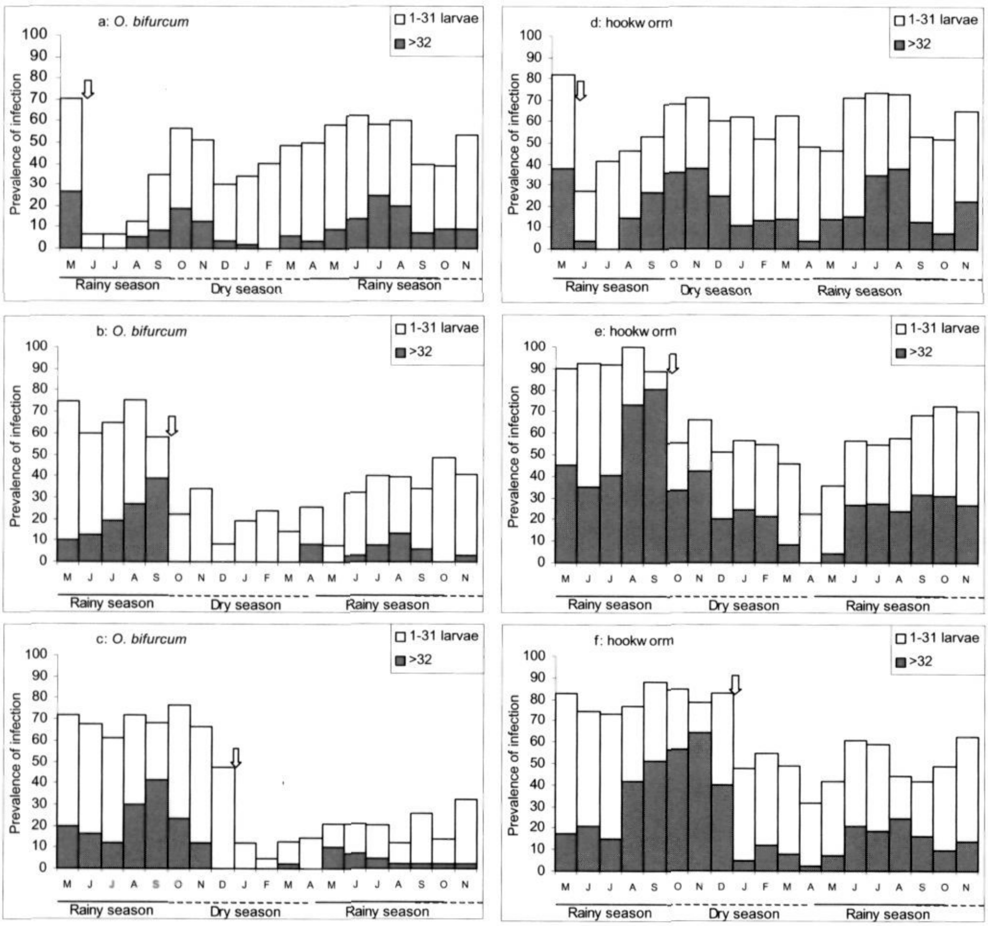

Fig. 3: Prevalence and intensity of infection and re-infection with O. bifurcum and hookworm in a population treated at the beginning of the rainy season (a, d), at the end of the rainy season (b, e), and in the middle of the dry season (c, f). The arrow indicates the moment of treatment.

bendazole was fairly effective in *Oesophagostomum* but significantly less so in hookworm, with average "cure rates" of 87% and 57% respectively. The "cures rates" are probably overestimates because of the fairly low sensitivity of the applied diagnostic methods used on one stool sample before and one after treatment (Pit *et al.*, 1999). The cures were lowest in the September intervention. This is not unexpected since infections were most intense during that time of the year and even a 95% kill of worms would result in appreciable numbers of "treatment failures", as described for schistosomiasis (De Vlas & Gryseels, 1992).

The comparatively low prevalence and intensity of infection during the dry season -as seen in the control group (figure 2)- may be due to either a temporarily low egg production of a stable parasite population or to an increasing mortality of the worms in the course of the dry season. Little is known of the seasonal fluctuations of surviving individual worms. The increase in egg excretion during the rainy season is probably not exclusively a reflection of recently acquired infections only, but also includes infections acquired during the previous transmission season, being either immature stages which developed to the egg producing phase or adult worms resuming egg production. Observations on experimental infections in monkeys showed that live larvae may be found in the tissues more than a year after exposure (Eberhard *et al*, submitted) and clinical observations in Ghana, too, indicate that larval stages may stay alive for many months, in the human host tissue. Adult worms of *Oesophagostomum sp.* may live up to 21 month after infection (Curtice, 1890), but not much is known about the effect of different seasons on the female egg production.

Intensity of infection tended to be slightly lower, both for *Oesophagostomum* and hookworm, during the 1996 rainy season compared to the season of 1995, which hamper somewhat the interpretation of the data depicted in figure 3. Superficial analysis shows that interpretation is not easy but at least one conclusion can be drawn from figure 3: increases in rates of larval-positive cultures, and more pronouncedly, increases in numbers of larvae counted, only occur during and shortly after the rains. It would appear that transmission of *Oesophagostomum* as well as hookworm is limited to the rainy season.

The prevalence of hookworm positive cultures as well as the hookworm larval counts increased rapidly in group 2 following the 1995 rains. Hookworm-reinfection in the subjects of groups 3 and 4 (treated after the 1995 rains) were somewhat lower, during the 1996 rains, as seen in *Oesophagostomum*. Indeed, transmission was comparatively weak for both nematodes during the 1996 rains. The data of the control group suggest that the fall in egg counts in the course of the dry season and the subsequent increase in hookworm larval counts during the 1996 rains was likely to be partly caused by an increased egg-output of the female worm rather than by increasing worm populations.

For *Oesophagostomum*, not only were cure rates much higher compared to hookworm, but also the effects of treatment during the different seasons were far more pronounced. While treatment before the onset of the rainy season (group 2) resulted in a rapid increase in egg excretion, both prevalence and intensity of infection were much lower in those individuals who were treated after the rains (group 3 and 4).

Recent observations of Storey *et al.* (submitted) indicated that Albendazole treatment does not only evacuate adult worms from the intestinal lumen but juvenile tissue dwelling stages are killed as well. These observations are in agreement with the effect of Fenbendazole on immature *O. dentatum* worms in pig (Praslicka *et al*, 1997). When treated after the transmission season (as in group 3 and 4) the rise in larval counts during the 1996 rains was probably mainly caused by newly acquired infections, although resumed egg excretion of some parasites which survived the treatment cannot be excluded. When treated before the (1995) rains, however, the rise in larval counts during the 1996 rains must be assumed to be the combined result of newly acquired infections and of mature infections derived from arrested larval development and increased egg excretion of surviving adult worms. Part of the infections acquired in 1995 is likely to develop only into mature egg producing adults after a period of arrested development. The observations suggest, but do not prove, that such mechanism of arrested larval development, so commonly seen in Strongyle infections of animals may occur in man as well.

The operational consequences of the observations are that treatment is most effectively given after the rains: it will take a long time before reinfection occurs and even during the next rainy season reinfection would seem to be slower as compared to situations where treatment was given before last year's rains. However, when albendazole is used in mass treatment, a higher dose should be used to improve the cure rates. Particularly for hookworm, the dosages used were not very satisfactory.

The data also indicate that transmission is variable, from one year to another. It indicates that the transmission system is pretty fragile and control may benefit from such fragility. At the same time, however, it must be realized that control trials must be followed for a number of years: the year to year fluctuations in transmission required long periods of evaluation.

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