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

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Chapter 1

General introduction

EARLY HISTORY

The first report of a human infection with *Oesophagostomum bifurcum* was published in 1905 by Railliet & Henry. They described six female immature worms that were found by Brumpt in 1902 when he performed autopsy on a 30-year old African man, who had been living near the river Omo, southern Ethiopia (Railliet & Henry, 1905; 1910). In the following decades a number of cases of human infections with *Oesophagostomum spp.* were reported from a diversity of locations in the world (Brazil, Nigeria, Indonesia, Zimbabwe, Uganda, etc...(Railliet & Henry, 1909; Leiper, 1911; Lie Kian Joe, 1949; Gordon *et al.*, 1969; Anthony & McAdam, 1972)). There was some confusion in the name of the species, but the taxonomic studies of Travassos & Vogelsang (1932), later summarised for the human infections by Chabaud & Larivière (1958), have resolved the debate. Today, three species are recognised to cause occasional infections in humans (*O. aculeatum, O. stephanostomum* and *O. bifurcum*). Because of the number of reports, human oesophagostomiasis has always been considered to be a rare zoonosis. In 1964, however, Haaf & van Soest, described nine human cases originating from Bawku (northern Ghana), and presumed, "the possibility that man himself may act as a source of infection, can not yet be discarded".

During the period 1980-1984, Dr S. Baeta, surgeon of the Regional Hospital of Dapaong (northern Togo), described a large number of patients (54) who presented with a visible tumor in the abdominal wall. Upon operation of these patients, it appeared that multiple nodules were found on the colon. Histological examination revealed that these nodules were in fact abscesses that contained an immature nematode, which could be identified as belonging to the genus *Oesophagostomum* (Gigase *et al.*, 1987).

In 1986, Prof. Gigase, of the Tropical Institute of Antwerp, and Dr Polderman from the Laboratory for Parasitology Leiden, traveled to northern Togo, to examine the possibilities for research on the occurrence of *Oesophagostomum* infection in humans. The following year, Dr Krepel went to Dapaong and started his research on the role of man in *Oesophagostomum* infection, which led to his thesis: "A study on the taxonomy, diagnosis, epidemiology and drug treatment of *Oesophagos-*

tomum bifurcum in northern Togo and Ghana" (Krepel, 1994).

STATE OF KNOWLEDGE

Genus and biology of the worm

Oesophagostomum bifurcum is an intestinal nematode, normally infecting monkeys only, but now commonly found among the human population of northern Togo and Ghana. Representatives of the genus *Oesophagostomum* are of veterinary importance; they can infect a wide range of animals, all over the world. The first *Oesophagostomum* species were discovered in cattle (*O. radiatum*) and pigs (*O. dentatum*) in 1803 (Levine, 1968). The name *Oesophagostomum* has its origin in the typical shape of the head, with an excretory pore (stoma) clearly visible in the ventral groove of the head at the level of the oesophagus (figure 1). The adult worms of *Oesophagostomum bifurcum* are straight roundworms, 8-17 mm long, tapering at both ends. The mouth is terminal and surrounded by an oral collar. The bursa copulatrix of the male and the straight and pointed tail of the female worm make it easy to distinguish the genders (Blotkamp *et al.* 1993).

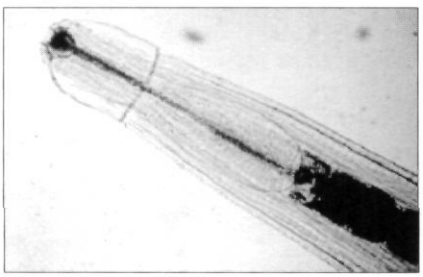

Fig. 1: Adult Oesophagostomum worm showing the typical ventral groove

Prevalence of infection

In the rural area of northern Togo and Ghana, a survey was made in which stool samples were collected and cultured. The prevalence of infection with *O. bifurcum* was often high but varied from one village to another, with an average of 30%. The highest prevalences were found in the rural villages, most distant from the main roads. The prevalence is higher in females than in males and comparatively low in children under the age of five. Although the route of infection remains obscure, *O. bifurcum* is a locally common parasite of humans, not requiring an animal reservoir for completion of its life cycle. In that same area 66% of the population is infected with hookworm (*Necator americanus*) (Polderman *et al,* 1991; Krepel *et al.,* 1992a).

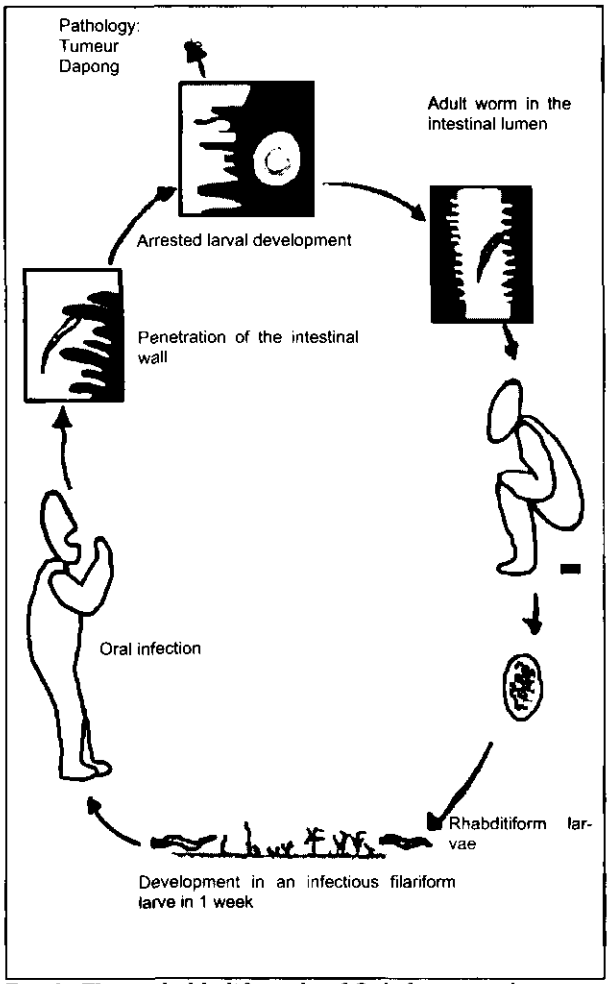

Fig. 2: The probable life cycle of O. bifurcum in humans

Life cycle

The normal and probably only route of infection with *Oesophagostomum* in animals is oral (Dash, 1973). Infective L3 larvae are swallowed while grazing or eating soil-contaminated food. In humans, an oral route of infection is most likely followed as well, since we did not succeed in establishing a percutanuous infection through the skin of a human volunteer. The probable life cycle is shown in figure 2. After ingestion of the larvae by the host, they penetrate the intestinal wall; here the larvae develop in young adult worms before re-entering the intestinal lumen to start egg produc-

tion. Some larvae might go in arrested larval development (ALD) and stay for a prolonged period of time in a nodule in the intestinal wall.

The calculated median egg production per female worm was 5 055 eggs/day, which is comparable with the production of other nematodes of the same superfamily (Krepel & Polderman, 1992b). The eggs are excreted with the stools of the host. Outside the host, in a moist environment, these eggs develop through two moults in third stage larvae. The development from freshly laid egg to infective larvae takes 4-7 days, depending on the environmental conditions. The infective larvae need to be ingested by a new host and the cycle is completed.

Pathology

Most infections of *O. bifurcum* are asymptomatic. But the disease is known by the local population as "Koun Koul", meaning, "turtle in the belly" and also as "Tumeur de Dapaong". Some *O. bifurcum* juveniles might develop in the colonic wall, causing pus-filled granulomas. The pathology has two distinct forms. Uninodular oesophagostomiasis presents as a painful abdominal mass, which may be visible on the abdomen of the patient (Figure 3). Multinodular oesophagostomiasis comprises hundreds of small nodules in the the wall of the large intestine (Storey *et al.*, 2000) Each nodule contains a dense infiltrate made up of eosinophils and macrophages and often an immature worm The intestinal wall is grossly thickened but mucosa and serosa are always intact. Microscopical examination shows that nodules are formed both between the mucosa and the muscle layer and between the muscle layer and the serosa.

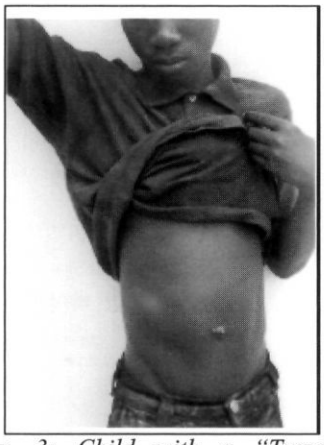

Fig. 3: Child with a "Tumeur de Dapaong"
(photo by Dr. N. Spannbrucker)

Sometimes the inflammatory reaction is extended to adhesions around the bowel causing serious complications such as intestinal obstruction (Gigase *et al.*, 1987). There are also cases described of ectopic lesions with nodules found in other places

than the intestine: on the omentum, in the liver or skin.

Morphology

The eggs of *O. bifurcum* are morphologically identical to those of hookworm: length and width are within the range given for hookworm (Figure 4).

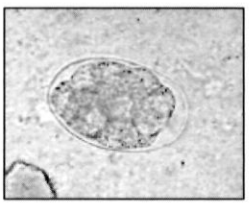

Fig. 4: egg of Oesophagostomum/ hookworm

The L_3 stage larvae of *Oesophagostomum bifurcum,* on the other hand, have typical morphological features, which makes it easy to distinguish them from L_3 stage hookworm larvae (Figure 5).

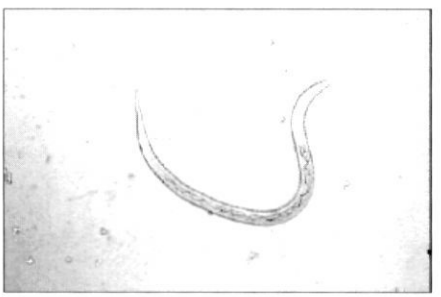

Figure 5: larvae of O. bifurcum

O. bifurcum larvae are longer than those of hookworm and Strongyloides, they have a long "hairlike" tail of sheath, the transverse striation of the sheath and the triangular intestinal cells are prominent. The number of cells (16-32) appears to be an unstable characteristic (Blotkamp *et al.*, 1993).

Diagnosis

Coproculture

Diagnosis of infections with intestinal nematodes is usually based on the identification of the eggs in the faeces of the patients. In this case the eggs of *O. bifurcum* are morphologically identical to those of hookworm. Therefore, on the basis of stool examination alone, it can not be concluded whether a person is infected with hookworm or *Oesophagostomum*. Only when hookworm-like eggs are allowed to develop into L_3 stage, is identification of the genus *Oesophagostomum* possible. To obtain these larvae the stools have to be cultured in a moist environment (Figure 6). For this 3 g of faeces is mixed with an equal quantity of vermiculite[1], divided in two and placed on moist filterpaper in two petri-dishes. Stools are cultured for a week and stirred every day to reduce

[1] Vermiculite: an altered mica that curls before the blowpipe flame and expands greatly at high temperature, forming a water-absorbent substance used in seed-planting, and also used as insulating material. (Chambers Dictionary).

the growth of fungi. Maggots are removed. Larvae migrate from the faeces to the clean water surrounding the filterpaper. On day 7, the culture fluid is poured off in a conical tube, the petri-dish is rinsed and the water added to the conical tube. After two hours of sedimentation, 100 µl of sediment is taken up with a micropipette and examined microscopically at low magnification (4x10), larvae are identified and counted by species.

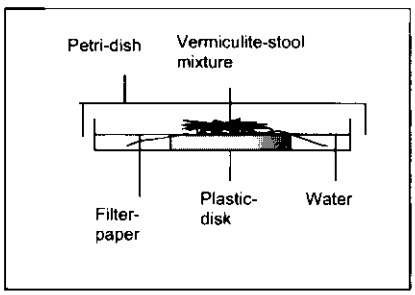

Fig. 6: schematic representation of the coproculture method

There was a highly significant correlation between egg counts and the combined number of *Oesophagostomum* and hookworm larvae (Krepel *et al.*, 1995a). Therefore the coproculture method can be used in a semi-quantitative way to indicate the intensity of infection.

Serology

Stool cultures are quite fastidious and require some practise to differentiate the larvae. Therefore a new tool based on the detection of parasite specific antibodies was utilised to diagnose infections in patients living in areas where hookworm-like eggs are commonly found in the stools. An IgG_4-specific Enzyme Linked Immunosorbent Assay (ELISA) was developed to diagnose human infections with *O. bifurcum*. However the precise sensitivity could not be determined and possible cross-reactivity between *O. bifurcum* and hookworm antibodies could not be excluded (Polderman *et al*, 1993).

Treatment

For the treatment of mixed *O. bifurcum* and hookworm infections in humans, different anthelmintics have been evaluated. Albendazole (400 mg) was most effective against both parasites, with a cure rate of 87% of the *O. bifurcum* infections and 61% of the hookworm infections. Pyrantel pamoate, 2x10mg/kg was effective against *O. bifurcum* (82% cured) but not against hookworm (18% cured). The cure rates for both parasites were moderate with thiabendazole, and poor with levamisole treatment (Krepel *et al.*, 1993). After chemotherapeutic intervention in the dry season, most subjects remain free of *O. bifurcum* infections until the following rainy season, when reinfec-

tion quickly occurs (Krepel *et al.*, 1995b).

Most patients with a "tumeur de Dapaong" are treated successfully with anti-inflammatory drugs, sometimes accompanied by antibiotics. Complications like intestinal perforation or bowel obstruction are always treated surgically.

AIMS OF THE RESEARCH PROJECT

In northern Togo *O. bifurcum* infections are not only widely distributed, but also the cause of significant morbidity and pathology known as "Tumeur de Dapaong". Krepel's thesis on human Oesophagostomiasis elucidated many aspects of this locally important health problem. Research on health issues in Togo is first of all meant to be problem-solving. The financial support of the Netherlands Foundation for the Advancement of Tropical Research (WOTRO) offered the possibility to continue a research project in northern Togo. Because of the existence of the well-furnished laboratory in the "Centre Hospitalier de Dapaong" and the good collaboration with the authorities of the Togolese government, the research project was continued in Dapaong.

In the case of the present Oesophagostomum project more detailed knowledge of the exact way of transmission and the biology of the parasite is required to successfully design strategies for prevention and control. Therefore one aim of the project was to follow-up an endemic population after treatment in different seasons, and to determine the exact geographical distribution of the parasite. Transmission with *O. bifurcum* is certainly influenced by the capacity of the larvae to survive extremely harsh condition. These abilities are experimentally assessed.

In neighbouring endemic regions in northern Ghana, P. Storey and collaborators made considerable progress, over the last few years, to arrive at a description and a classification of the clinical cases in a hospital setting and at the recognition of preclinical cases at the community level. Ultrasound appeared a helpful tool for population based studies of pathology. Measurement of morbidity and assessment of the clinical impact of intervention became within reach.

In addition to the clinical assessment of the impact of intervention, which is likely to be time consuming and difficult to quantify, parasitological methods to follow intervention need

to be worked out in greater detail. Diagnostic approaches have to be improved and the variability of the diagnostic parameters has to be assessed.

It must be realised that excretion of eggs and counting larvae in stool cultures is linked to the presence of mature lumen-dwelling worms but pathology is caused by larval stages that are still in the process of development to adulthood. It is likely, therefore, that clinical cases may remain unnoticed and alternative serological methods may recognise infections that are missed with parasitological methods. Although previous studies were based on measuring specific anti-Oesophagostomum IgG4 antibodies, this method has not been practically used on any scale. Further experience with such methods is discussed and attempts to further improve on serodiagnosis are therefore an essential part of the present study. Yet another alternative is a molecular approach in which species specific DNA is detected with PCR.

Up to date little is known about the parasite-specific cellular immune response in humans chronically infected with *O. bifurcum* and *N. americanus*, and about the factors and mechanisms contributing to resistance. One additional aim of this study was to determine the expression of immunity in humans chronically infected with *O. bifurcum*.

Each of the following chapters elaborates on the various aspect of the research on *O. bifurcum* infection in humans in northern Togo. The thesis is concluded by a general discussion of the implications of our findings on human Oesophagostomiasis.

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