

## SUMMARY

During the twentieth century, occasional cases of human infections with *Oesophagostomum bifurcum* have been reported in the medical literature and were, therefore, considered as rare zoonoses. However, in northern Togo and Ghana, human oesophagostomiasis was recognised to be endemic. Most infections are asymptomatic but occasionally juvenile *O. bifurcum* worms encapsulate into the colonic wall, causing granulomatous pus-filled nodules, which may adhere to the surrounding tissues and form a mass. Sometimes this mass is clearly visible on the patient's abdomen. The disease is locally known as a "Tumeur de Dapaong". Studies on several domestic animals have shown that the life cycle of *Oesophagostomum* does not require an intermediate host and transmission is oral. The life cycle and route of transmission of the parasite in man should be similar.

Detailed maps of the geographical distribution of *O. bifurcum* and hookworm (*N. americanus*) infections in northern Togo are presented in chapter 2. Thirty percent (100 000 individuals) of the population is infected with *O. bifurcum* and more than 70% (230 000 individuals) with hookworm. Villages with high *O.*

*bifurcum* prevalence are confined to a number of foci. All villages examined are infected with hookworm. Women are more often infected with *O. bifurcum* than men, while the converse is true hookworm. The prevalence and intensity of infection with both parasites are clearly age dependent, with 10 to 14 years old children bearing most of the parasite load.

For parasite specific diagnosis of *O. bifurcum* and hookworm infections, a coproculture is required, because only the infective larvae, and not the eggs can be distinguished morphologically. The sensitivity of this method is above 90% for duplicate coprocultures, but larval counts vary considerably from day to day for the same patient, and from one samples to another for the same stool. Therefore, for the proper assessment of infection intensity, repeated examination on the same stool samples is recommended. Stool collection of new samples on subsequent days does not considerably add to the sensitivity of the coproculture method, as compared to repeated exams on one sample. (Chapter 3). The coproculture has additional shortcomings, i.e. it can only be performed on fresh stool samples and it is quite fastidious. Moreover, *O. bifurcum* induced pathology is not caused by the lu-

men-dwelling egg-laying worm but by the migrating and highly immunogenic larvae encapsulated in the intestinal wall. In chapter 4 the use of an alternative diagnosis based on the detection of parasite specific antibodies in the sera of patients, is evaluated. *O. bifurcum* specific IgG<sub>4</sub> antibodies are measured in patient-sera from northern Togo as well as in patient-sera from central Togo (where *O. bifurcum* is not endemic). Pre-absorption of the sera with *N. americanus* coated beads demonstrated that there is some cross-reactivity between *N. americanus*-specific IgG<sub>4</sub>-antibodies and *O. bifurcum* antigen. The detection of IgE antibodies against *O. bifurcum* and hookworm is more specific, but not sensitive enough to detect all infections and therefore needs further improvement.

In chapter 5 the applicability of specific PCR's to amplify DNA from fecal samples is evaluated, as an alternative method for the differential diagnosis of *O. bifurcum* and hookworm. The PCR does not show non-specific amplification with a range of control DNA samples. The *O. bifurcum* PCR amplifies specific *O. bifurcum* products of  $\approx 220$  bp from 57/61 fecal samples known to contain *O. bifurcum* L3 larvae after coproculture. The *N. americanus*

PCR amplifies specific *N. americanus* products of  $\approx 250$  bp from 137/145 fecal samples known to contain *N. americanus* L3 larvae. Moreover, PCR detects 26 additional *O. bifurcum* cases in 72 samples in which no *O. bifurcum* larvae are found and 46 *N. americanus* cases in 79 samples where no *N. americanus* larvae are found after coproculture. No *O. bifurcum* DNA is detected in 91 stool samples from individuals from two non-endemic villages. Therefore, PCR can be used as a powerful tool to provide information about the presence or absence of *O. bifurcum* and *N. americanus* infections in a population.

In order to understand the mechanism of transmission, the seasonal changes in larval counts have been carefully monitored in groups of subjects treated before and after the rains, as described in chapter 6. Larval counts varied considerably from one rainy season to the other. Albendazole has high cure rates for *Oesophagostomum* but rather modest ones for hookworm infections. Treatment of population groups in different seasons of the year shows that reinfection is confined to the rainy season. Following treatment after the rains, the larval counts remain low until the following rainy season. This distinct pattern of *O.*

*bifurcum* transmission should be considered when designing control programs.

In chapter 7, *Oesophagostomum* larvae cultured from human stools, unlike the larvae of *Necator americanus*, are shown to survive desiccation for at least six months. In addition, 93% of the *O. bifurcum* larvae frozen for 24 hours at  $-15^{\circ}\text{C}$  regain motility when returned to ambient temperatures. Desiccated larvae can even be rehydrated in an artificial mixture made to resemble human gastric juices, indicating the possibility of dust-born infections. Such sturdiness is likely to contribute to the intense transmission in northern Togo and Ghana.

Chapter 8 investigates parasite-specific cellular reactivity and Th1- or Th2-type cytokine responses in humans infected with *Oesophagostomum bifurcum* and/or *Necator americanus*. Cellular responses are not strictly dominated by type 1 or type 2 T helper cell reactivity. In co-infected patients cellular hyporesponsiveness to parasite antigens is observed, but enhanced production of TNF- $\alpha$  and IFN- $\gamma$  can also be measured. Th2-type cytokines (IL-5 and IL-10) are produced in equal amounts by PBMC from individuals with mono- and co-infections.

The research described in this thesis has shown that *O. bifurcum* is a common intestinal parasite of the population of northern Togo. There are good diagnostic methods i.e. coproculture, parasite specific ELISA and PCR. In addition, longitudinal studies have shown that transmission of both *O. bifurcum* and hookworm can be reduced when treatment is given at the beginning of the dry season.

