Controlling human Oesophagostomiasis in Northern Ghana
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-Chapter 3-

Oesophagostomum bifurcum-induced nodular pathology in a highly endemic area of Northern Ghana

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Transactions of the Royal Society of Tropical Medicine and Hygiene
99, 417-22 (2005).

Abstract

Human infection with *Oesophagostomum bifurcum* is rare globally, but focally endemic and common in Ghana and Togo. Two clinical presentations are identified: uni-nodular disease, which may be recognized as a 'Dapaong Tumour', and multi-nodular disease. Here, we describe the prevalence of *O. bifurcum* infection and the association with nodular pathology in northern Ghana. The study was performed in October 2001. Out of a well-defined population of approximately 18,000, 928 subjects of all ages were randomly selected for parasitological and ultrasound examination. In stool cultures, 44% had detectable third-stage *O. bifurcum* larvae present. Females were more often infected than males ($P < 0.05$). In 34% of the samples, nodules were detected along the colon wall, with the ascending and the transverse colon being the most affected regions. Significant correlations existed between the intensity of infection and the presence of nodules, both at the village and the individual level ($P < 0.001$ for both). Patients with multi-nodular pathology had significantly higher larval counts than patients with uni-nodular pathology. The present data suggest that nodular pathology, and probably the severity of the disease, are directly related to intensity of the infection.

Introduction

Human oesophagostomiasis, normally due to infection with *Oesophagostomum bifurcum*, is rare in most parts of the world except in the north eastern part of Ghana and in northern Togo where it is common (Polderman *et al*., 1991). The prevalence and distribution of infection determined by coproculture have been well described for the area; it is estimated that a quarter of a million people are infected, with one million more at risk (Pit *et al*., 1999a; Polderman *et al*., 1999).

The biology of transmission of this nematode is poorly understood but infection is presumably by oral ingestion of the L_3- infective larvae. Ingested larvae of related species of veterinary importance are believed to assume a histotropic phase of development in the colon wall and finally return to the lumen of the intestine to continue development into adult reproductive stages (Dash, 1973). Eggs produced by the female adult worm are passed with stool, and when deposited in conditions of favourable soil temperature and humidity, they normally hatch and develop into infective forms ready to infect another host within seven days.

During the histotropic stage, nodules develop in the colon wall and these can be identified using ultrasonography. Occasionally, larvae migrate to extra intestinal sites such as the abdominal walls where nodules may occur as solitary, palpable and painful protruding masses. The palpable inflammatory mass around the larvae is usually referred to as a 'Dapaong Tumour' (Storey *et al*., 2000a). In others, invasion of the colon wall by large numbers of L_3-larvae results in the development of many pea sized, pus-filled, worm-containing nodular lesions, characterized by a grossly thickened and oedematous submucosa and subserosal layers of the colon (Storey *et al*., 2000b).

It so far remains unknown whether the development of pathology is first of all an expression of an extreme immune response in a particular sub-population of infected subjects or a normal consequence of infection. In the present paper, we identify and describe the association between infection and pathology and discuss the extent to which *O. bifurcum* infections induce nodular pathology in a highly endemic area of northern Ghana. The different types of subclinical nodular pathology are described in detail.

Materials and methods

Study area and subject selection

The study was conducted in Garu area within the Bawku East district of the Upper East Region of Ghana in October 2001 (latitude 10.50ºN, longitude 0.50ºN). The inhabitants of this area are of five ethnic origins: Kusasi, Mamprusi, Fulani, Busanga and the majority Bimoba. The study area covers about 150 (10×15) km^2 along the Ghana-Togo border and comprises 29 villages. The approximately 18,000 inhabitants of the study area live in 1570 compounds (mud-built structures for human dwelling). All villages and compounds were mapped with the aid of GPS version 12 (GPS 12, Garmin International, Inc., Olathe, KS 66062 USA) and all villages, compounds and individuals were registered and assigned unique identification numbers as civil registries are not available in the area.

From each of the villages, we randomly selected 10% of the compounds to be included in the study. From the 142 compounds selected, 1476 individuals were available and after verbal explanation of the study by local interpreters, they were invited to submit stool samples for examination. A total of 1314 (89%) persons provided stool samples, which were collected in labelled plastic containers. Coprocultures were performed in duplicate from each sample on the day of collection, as previously described (Polderman *et al*., 1991). After five to seven days of culture, larvae were microscopically identified at low magnification (100 x) and counted. The characteristic third-stage larvae of *O. bifurcum* were distinguished from those of hookworm and *Strongyloides* spp. (Blotkamp *et al*., 1993).

All individuals for whom stool samples were available were invited to visit our mobile field clinic for clinical examination. Of the 1314 subjects for whom stool culture results were available, 928 subjects (71%) were examined. The rest were either absent or too old and weak to visit the ultrasound clinic. The examination consisted of bimanual palpation of the abdomen to elicit masses suspected to be *O. bifurcum*-induced nodular lesions. Subsequently, a Kretz technology LS portable ultrasound machine (Kretztechnik AG,Teifenbach, Austria), equipped with a 3.5-4.5 MHz linear transducer, was used to scan the abdomen with the patient in the supine position. A general abdominal survey was performed systematically with the liver and spleen as landmarks, looking for nodular pathology in both the abdominal walls and the colon. Images of all detectable nodules were observed in both transverse and longitudinal planes to describe the structure and to avoid misdiagnosis from tubular structures.

Location of nodules in the various abdominal regions, type, number per person and appearance were described and recorded on a printer and with a digital camera. Ultrasound examination was performed without prior knowledge of the parasitological status of the individual to minimize bias. Uni-nodular pathology was defined as a solitary nodule of well-defined ovoid shape, circumscribed by a thin wall located in only one region of the colon whereas multi-nodular pathology was characterized by several solitary nodules or a cluster of nodules.

Ethical clearance for the study was obtained from the Ministry of Health of Ghana and a witnessed informed consent procedure was followed. Both the head of the household and the subjects enlisted for both parasitological and clinical examinations were verbally asked for their consent; another team member kept a record of proper adherence to this verbal procedure. After the survey all persons examined were treated with 400 mg albendazole (Zentel®, GlaxoSmithKline Pharmaceuticals, Brentford, UK). The project was approved and recommended by the Danish Central Scientific-Ethical Committee of Denmark.

Data analysis

The demographic, parasitological, clinical and ultrasound data were entered in a computer using EpiInfo, version 6 (CDC, Atlanta, GA, USA). Statistical analysis was performed by SPSS, version 10 (SPSS Inc., Chicago, IL, USA). Infection and pathological lesions were expressed as prevalence figures, stratified by age and gender at the individual level. Larval counts and nodular pathology were presented as medians with corresponding interquartile ranges (IQR) as data were not normally distributed. Differences between groups were analysed with non-parametric tests (X^2, Kruskal-Wallis). The association between intensity of infection and nodular pathology was analysed by cross-tabulation at an individual level, and by regression at a village level. A *P*-value of less than 0.05 was used to imply statistical significance for all tests.

Results

Data on both stool cultures and ultrasound examination of the abdomen from 928 subjects (453 males, 475 females) were available for analysis. Their estimated ages ranged from 0 to 95 years with a median age of 13 years (IQR 6-35 years). On examination, 404 (44%) had third-stage *O. bifurcum* larvae detected (median count one per double culture, i.e. 4 g of faeces). Prevalences of infection by age group and gender are shown in Table 3.1. Females were significantly more often infected than males (47% vs. 40%, P = 0.026). On ultrasound examination, 314 (34%) subjects had at least one *O. bifurcum*-induced anechogenic nodular lesion detected (Table 3.1). The proportion of persons with nodular pathology was also higher in females compared with males (36% vs. 31%, P = 0.05).

The distribution of nodular pathology is shown in Table 3.2. Nodular lesions mostly occur in the colon ascendens and transversum. Only a few lesions were located in the colon decendens and none in the colon sigmoideum.

The distribution of nodules in the colon was the same for the various clinical presentations.

Table 3.1: Prevalence of *O. bifurcum* infection and nodular pathology by age and sex

Age group	Number examined			% infected			% with nodular pathology		
	Total	male	female	**total**	male	female	**total**	male	female
< 5	**151**	69	82	**33**	36	29	**32**	30	33
5-9	**215**	125	90	**51**	49	54	**43**	44	42
10-14	**121**	69	52	**41**	42	40	**30**	28	33
15-19	**67**	42	25	**37**	36	40	**33**	29	40
20-29	**82**	26	56	**42**	31	46	**39**	31	43
30-39	**89**	26	63	**49**	39	54	**32**	23	35
>=40	**203**	96	107	**45**	35	54	**27**	21	33
total	**928**	453	475	**44**	40	47*	**34**	31	36*

*Females compared to males test $p < 0.05$

Table 3.2: Description of nodular pathology

Location	Uni-nodular pathology (n= 144)	Multi-nodular pathology Uni-location (n=17)	Multi-location (n=153)
Ascending colon	102 (70)	12 (71)	226 (61)
Transverse colon	31 (21)	3 (18)	91 (24)
Descending colon	12 (8)	2 (12)	56 (15)
Sigmoid colon	0 (0)	0 (0)	0 (0)
Any location	145 (100)	17 (100)	373 (100)*

Data are presented as numbers (%) *Numbers add up to more than the maximum of 314 since pathology may occur in the same person at more than one location.
The distribution of nodules was not different between the various clinical presentations

Table 3.3 shows that among the participants with nodular pathology, 145 (46%) had only one nodule detected (median maximum nodular diameter 13mm [IQR 10-17 mm]. A majority of 152 (49%) participants, however, had multiple nodular pathologies detected at different locations with a median maximal nodular diameter of 15mm (IQR 13-18 mm). In a minority of participants, 17 (5%), multiple nodular pathologies were detected at only one location, the median maximal nodular diameter being 13mm (IQR 8-17 mm). The differences in median maximal nodular diameter between the groups were statistically highly significant ($P = 0.001$).

When we compared larval counts in patients with no pathology, uni-nodular pathology, and multinodular pathology at one or several locations, we found the median counts to be significantly different: 0 (IQR 0-3), 1 (IQR 0-11.5), 0 (IQR 0-17) and 9 (IQR 0-33) respectively ($P < 0.001$). The distribution is presented in Table 3.3. This implies that subjects who excrete high numbers of larvae have the highest risk of developing multi-nodular pathology at several locations.

Table 3.3: Severity of nodular pathology in relation to the intensity of infection

Larval count	No pathology n=614	Uni-nodular pathology n=144	Multi-nodular pathology Uni-location n=17	Multi-nodular pathology Multi-location n=153
0	402 (66)	71 (49)	10 (59)	41 (27)
1-9	121 (20)	36 (25)	3 (18)	39 (26)
10-32	52 (9)	18 (12)	2 (12)	32 (21)
33-100	30 (5)	14 (10)	1 (6)	19 (13)
>100	9 (2)	6 (4)	1 (6)	21 (14)

Data are presented as numbers (%)
Severity of nodular pathology compared to intensity of infection $p<0.001$

The association between the prevalence of *O. bifurcum* detected by stool culture and nodular pathology at the village level is presented in Figure 3.2**.** Nodular pathology dependent on *Oesophagostomum bifurcum* count by gender in study subjects in northern Ghana, October 2001. Prevalence of nodular pathology in males (solid bars) and females (hatched bars). The increase of nodular pathology at higher larval counts was statistically significant for males and females (Kruskal—Wallis, $P < 0.001$ for both) Figure 3.1. There was a statistically highly significant correlation ($P = 0.001$) at the village level. Only one village could be considered as an outlier: nodular pathology was seen in 90% while the corresponding prevalence was only 10%.

At the individual level, cross-tabulation of any infection with any nodular pathology provided an odds ratio (OR) of 3.02 (95% CI 2.9—3.1, $P = 0.001$), indicating that those with larvae detected in their stools had a three-fold increased risk of having nodular pathology diagnosed at ultrasound examination. The risk was considerably higher when the intensity of infection was higher. Over 80% of those with more than 100 larvae detected had nodular pathology (Figure 3.2). At the same time Figure 3.2 and Table 3.3 show that nodular pathology is not limited to stool culture positive subjects.

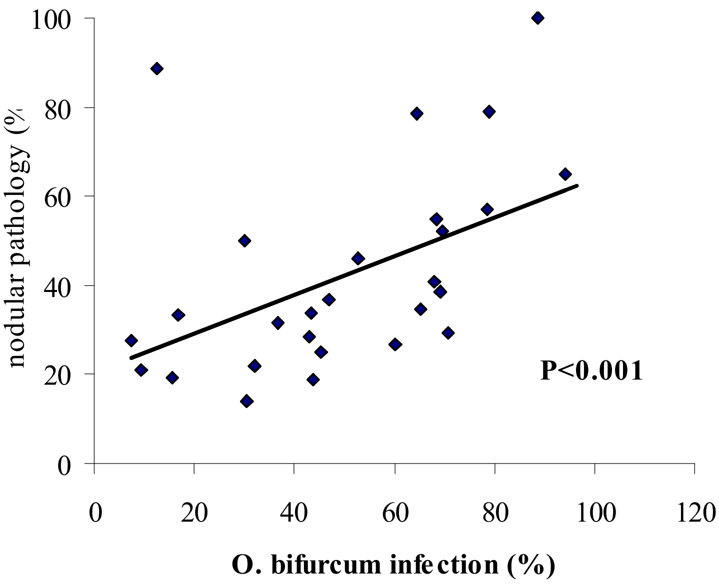

Figure 3.1: Association between *O. bifurcum* infection and nodular pathology at a village level.

Discussion

The main findings of this study are in line with our earlier, preliminary observations based on a small-scale study in the village of Sanakpesir, located in the same area, and area-wide surveys of *O. bifurcum* in northern Ghana and Togo (Pit *et al*., 1999a; Yelifari *et al*., 2005). Village-specific prevalences of infection in this study are considerably higher than those described by Yelifari *et al*. (2005). Their study included many villages with no or few cases of infection and stool examinations were based on single stool cultures only, resulting in considerably lower diagnostic sensitivity. No attempts were made to record intestinal pathology in that study. Storey *et al*. (2000d) did use ultrasound examination of the abdomen, in combination with stool cultures, but their sample size was too small for appropriate analysis. The present study thus expands on the observations of those preliminary studies.

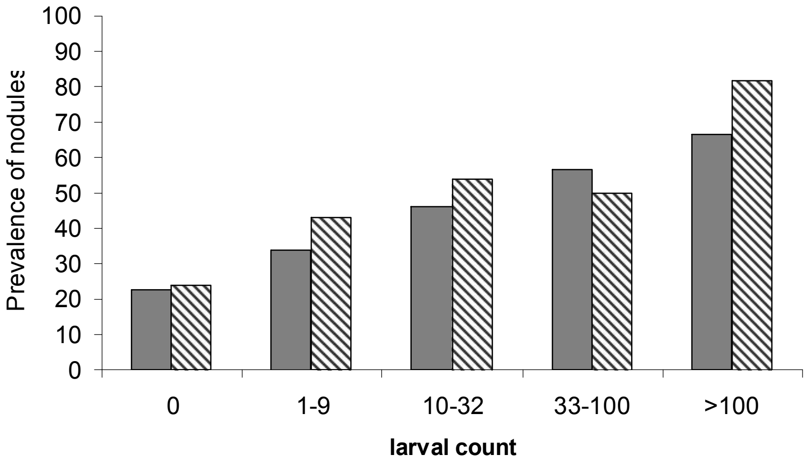

Figure 3.2. Nodular pathology dependent on *O. bifurcum* count by gender. Solid bars indicate prevalence of nodular pathology in males and hatched bars indicate that for females. The increase of nodular pathology at higher larval counts was statistically significant for males and females (both Kruskall-Wallis $p<0.001$).

At the population level, there was a strong correlation between the prevalence of infection and that of nodular pathology except in a single village where prevalence of nodular pathology was nine-fold higher than the prevalence of *O. bifurcum* infection. However, only 10 persons were examined in that village suggesting that this outlying finding was not important. At the individual level also, a correlation exists between stool culture results and ultrasound observations. Individuals who excrete *O. bifurcum* eggs had a three-fold increased risk of having nodular pathology.

Before analysing the relationship between positivity in stool culture and in ultrasound, it is important to note that both methods have their shortcomings and both are of moderate sensitivity. In stool culture, some light infections are easily missed, as demonstrated by Pit *et al.* (1999b). In the present study also, the comparative insensitivity of the stool culture procedure is evident from the fact that

7% of the culture-positive cases would have been missed if only one instead of two cultures had been examined (data not shown). As to the sensitivity of ultrasound, it should be realized that an estimated one-third of all nodules located in the colon cannot be detected by ultrasound due to sound extinction in that region. Moreover, in adult patients, wrinkled skin folds and fatty abdominal wall layers and larger abdominal cavities hinder investigation. The comparatively low proportion of subjects with nodular pathology in men and women aged more than 30 years, as demonstrated in Table 3.1, should probably be interpreted in this way.

Storey *et al*. (2000d) were the first to use ultrasound to explore the clinical outcome of *O. bifurcum* infection: they were unable to recognize a statistically significant correlation between infection and the presence of nodules. The absence of such a correlation, in their comparatively small-scale study, was explained in a biological way: the presence of nodular pathology represents the developing larva's inability to return to the host's colonic lumen and to mature and produce eggs. The presence of eggs in the host's stools, on the other hand, implies that larvae did successfully complete their life cycle and that they did not get stuck in the intestinal wall to cause the characteristic *O. bifurcum* nodules. In the present study, the statistically significant association between the presence of larvae, representing the presence of mature adult worms, and of nodular pathology, representing the larva's failure to develop into mature worms, indicates that the hypothesis put forward by Storey and co-workers may be correct only in occasional situations but not at the population level for the particular area studied. This means that even if some nodules develop in a human host, the majority of larvae are able to return to the intestinal lumen successfully.

The observed close correlation between culture and ultrasound positivity is further confirmed when considered quantitatively: the chance of developing nodules increases with increasing intensity of infection. The increase, however, is not linear, implying that, in heavier infections, a comparatively low proportion of larvae are caught in the nodular lesions in the intestinal wall.

This study has shown that the risks of developing ultrasound-visible pathology, as well as the severity of pathological presentation, do increase with increasing worm loads (i.e. with increasing numbers of larvae found in cultures). Among the lightly infected cases, visible pathology was seen in only about a third of patients, with more than half of them developing multi-location multi-nodular pathology. In the heavily infected cases, however, pathology was seen in two-thirds of the patients, with yet another two-thirds of these infected individuals developing multi-location multinodular pathology.

Until recently, it has been unclear whether clinical oesophagostomiasis should first of all be considered as an immunological overreaction in particular patients independent of the intensity of infection. The data presented in this study suggest that the degree of pathology is primarily associated with the numbers of eggs excreted and thus with the intensity of infection. The association of clinical disease with the intensity of infection remains likely but requires confirmation.

Conflicts of interest statement

The authors have no conflict of interest concerning the work reported in this paper.

Acknowledgements

The DBL-Institute for Health Research and Development, GlaxoSmithKline, Leiden University Medical Center (LUMC) and the Leiden-based Gratama Foundation provided funds for the fieldwork. Bayita Albano, Daniel Laar, Leonard Yelifari, Mohammed Awel, Moses Kolan and Mathilda Abugri made the fieldwork possible. In addition, we are most grateful to the following Dutch students for their assistance in the field and laboratory work: Bram Diederen, Gabrielle van Ramshorst, Esther Hamoen and Rutger van der Meer. The Ghana Health Service in Bolgatanga and Bawku are gratefully acknowledged for their support.