Chapter 4

EGG PRODUCTION OF *OESOPHAGOSTOMUM BIFURCUM*,
A LOCALLY COMMON PARASITE OF HUMANS IN TOGO

H.P. Krepel and A.M. Polderman

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In northern Togo and northeastern Ghana, *Oesophagostomum bifurcum* is a common parasite in humans. Diagnosis is based on coproculture because the eggs of hookworm and *Oesophagostomum* are indistinguishable. To determine the level of egg production, 12 subjects were treated with 2 x 10 mg/kg of pyrantel pamoate and the worms they evacuated were then counted. Pretreatment and post-treatment species-specific egg counts were calculated on the basis of larval and total egg counts. The median worm burden was 81 (range 12-300) per person. The calculated median egg production was 33.7 egg/gram of feces per female worm. Assuming a total daily stool production of 150 g/day, this amounts to 5 055 eggs/day, which is comparable with the production of other nematodes of the same superfamily.
Introduction

In animals, oesophagostomiasis is a common infection that causes serious pathology. It is caused by *Oesophagostomum* species, which are nematodes of the same superfamily as hookworm. Some examples are *O. colombianum*, *O. radiatum* and *O. dentatum*. It has been established only recently that in northern Togo and northeastern Ghana, it is also a common infection in humans. Using a coproculture method, third-stage *Oesophagostomum* larvae were found in 30% of the population [1]. Use of a coproculture method is essential, since the eggs of *Oesophagostomum* cannot be distinguished from those of hookworm by size or by morphological criteria [2]. In contrast, the third-stage, infective larvae are very different [3]. The level of egg production of the species involved, *O. bifurcum*, is not known. The egg production of *O. radiatum* is 5 000/worm/day [4]. Hookworm produces between 10 000 and 30 000/worm/day in humans, depending on the species and the worm burden [2,5]. *Ternidens deminutus*, another species belonging to the same order as hookworm and *Oesophagostomum*, produces between 3 500 and 7 000 eggs/day [6].

The purpose of this study was to estimate egg production of *O. bifurcum* in humans. Specific egg counts for *Oesophagostomum* before and after treatment were calculated using egg and larval counts. All adult worms excreted after treatment were counted. To avoid the possibility of quick degeneration of the adult worms after treatment, the participants were treated with pyrantel pamoate and a purgative.

Materials and methods

Trial population

In the village of Lotogou, about 25 km west of the main town of Dapaong, several households were visited. The inhabitants were screened using one coproculture. Persons who were positive for *Oesophagostomum* could participate provided they were five years of age or older and had not received anthelmintic treatment in the recent past. Pregnant women and subjects suffering with major illnesses were excluded.

Counting techniques

One to three days before treatment, two stool samples were collected. From each sample, two egg counts (Kato smears, 25 mg) and three stool cultures (classic charcoal method[1]) were done. After seven days, the numbers of both hookworm and *Oesophagostomum* larvae in each culture were identified according to the key of Little [3], and counted. One week after treatment, two egg counts and three coprocultures were performed on one stool sample.
Table 1. Egg production of Oesophagostomum bifurcum in 12 subjects in this study *

<table>
<thead>
<tr>
<th>Subject/sex</th>
<th>Pre-treatment</th>
<th>Worms</th>
<th>Egg production</th>
</tr>
</thead>
<tbody>
<tr>
<td>age(years)</td>
<td>epg</td>
<td>Mwl</td>
<td>Oel</td>
</tr>
<tr>
<td>1/F/9</td>
<td>4 360</td>
<td>17</td>
<td>89</td>
</tr>
<tr>
<td>2/F/10</td>
<td>2 160</td>
<td>37</td>
<td>56</td>
</tr>
<tr>
<td>3/F/25</td>
<td>1 840</td>
<td>64</td>
<td>117</td>
</tr>
<tr>
<td>4/F/40</td>
<td>10 180</td>
<td>391</td>
<td>28</td>
</tr>
<tr>
<td>5/M/8</td>
<td>2 600</td>
<td>249</td>
<td>28</td>
</tr>
<tr>
<td>6/M/8</td>
<td>420</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>7/M/8</td>
<td>1 180</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>8/M/8</td>
<td>9 140</td>
<td>164</td>
<td>241</td>
</tr>
<tr>
<td>9/M/8</td>
<td>2 540</td>
<td>62</td>
<td>221</td>
</tr>
<tr>
<td>10/M/9</td>
<td>3 440</td>
<td>63</td>
<td>61</td>
</tr>
<tr>
<td>11/M/12</td>
<td>4 580</td>
<td>107</td>
<td>173</td>
</tr>
<tr>
<td>12/M/70</td>
<td>9 120</td>
<td>217</td>
<td>12</td>
</tr>
<tr>
<td>Median</td>
<td>3 020</td>
<td>1 199</td>
<td>20,0</td>
</tr>
</tbody>
</table>

* Epg = epg/gram of feces; Hwl = hookworm larval counts; Oel = Oesophagostomum larval counts.

**Treatment and isolation of adult worms**

A purgative was given the day before treatment (d0) at 6:00 PM. The next day (d1) at 7:00 AM, participants were treated with 10 mg/kg pyrantel pamoate. Two hours after treatment, a purgative was given again. Treatment and purgation were repeated on the second day (d2) at the same times. All stools produced during day 1 and day 2 were collected. The stools were washed and sieved with 1-mm and 300-μm mesh sieves, and the adult worms were identified and counted.

**Calculation methods**

For the calculation of the worm's level of egg production, only data from those patients were included on whom Kato smears, stool cultures, and treatment were successfully performed. Moreover, very light infections (<10 worms evacuated) were excluded. Of the 12 cases meeting the above criteria, the arithmetic means of the pre-treatment egg counts (eggs/gram of feces [epg] in Table 1) were calculated for each individual. Using the larval counts for both hookworm (Hwl) and Oesophagostomum (Oel), it was calculated which part of the egg output was attributed to Oesophagostomum (Oe-epg) and hookworm, respectively. These
species-specific egg counts were then divided by the total number of adult worms to determine egg production per worm per gram of feces (epg/w), or by the number of adult female worms only to determine egg production per female worm per gram of feces (epg/fw).

**Results**

**Pretreatment counts**
The median larval counts were 59 (range 12 - 241) per culture for *Oesophagostomum*, and 64 (range 16 - 391) for hookworm. The median total egg count was 3 020 epg (range: 420 - 10 180), while the median for *Oesophagostomum* was calculated at 1 199 (range 226 - 5 443) epg (Table 1).

**Recovery of adult worms**
A total of 703 female and 562 male *Oesophagostomum* worms were counted. The number of worms per participant varied from 12 to 300 (Table 1).

**Calculation of egg production**
The calculated median level of egg production for one *Oesophagostomum* worm was 17.9 epg per worm, while for female worms, this figure was 33.7 epg per female worm (Table 1). Assuming a total stool production of 150 g/day, this would mean that the total daily egg output is 2 685 eggs/worm, and 5 055 eggs/female worm. The pretreatment species-specific egg counts as well as the larval counts of *Oesophagostomum* showed a good correlation with the observed worm burden (Figure 1).

**Discussion**
It has only recently been established that human infections with *O. bifurcum* are common in northern Togo and northeastern Ghana [1]. Many biological and medical aspects still have to be examined, one of them being the the level of egg production. Knowing the level of egg production of the parasite provides also a means of estimating the worm burden.

The morphological similarity of *Oesophagostomum* and hookworm eggs makes specific egg counts difficult to achieve. We used the proportion of *Oesophagostomum* versus hookworm larvae found in the coprocultures to calculate the number of *Oesophagostomum* eggs in the total egg counts.
Figure 1. Relationship between *Oesophagostomum* worm burden and larval counts (a) \( R = 0.91, P < 0.01 \) and specific egg counts (b) \( R = 0.94, P < 0.01 \).
Egg production

The median level of egg production was calculated to be 33.7 epg/female worm. Assuming a total daily stool production of 150 g, this comes to 5,055 eggs/day. These levels are consistent with those of hookworm and *Ternidens diminutus* [2,5,6].

It must be stressed that the calculations are based on the assumption that the development of hookworm and *Oesophagostomum* larvae in coprocultures is equally successful. The linear relationship between worm burden and larval counts, and worm burden and species-specific egg counts would indicate that this parity was probably the case in our study.

The mean egg excretion per female worm was calculated for 12 cases of an initial series of 30. Of the remaining 18 subjects, four were not included because live *Oesophagostomum* larvae were cultured following treatment. In five cases, there were indications that the pretreatment cultures were unreliable (few larvae cultured in patients excreting many eggs), while in nine cases, less than 10 adult worms were evacuated after treatment. In seven of the latter patients, very few eggs were found; these patients apparently had very light infections. In the remaining two, only a minor proportion of the feces produced after treatment was submitted for examination. It cannot be excluded that there is a bias in the resulting calculations of egg production; in very light infections, the level of egg production might be different.

After treatment with pyrantel pamoate 2 x 10 mg/kg, few adult specimens of *Necator americanus* were recovered. One week after treatment, *Oesophagostomum*-positive cultures (excluded from the analysis in this report) were observed in only four of 30 persons. Hookworm larvae, however, were found in 28 cases. Thus, treatment with pyrantel pamoate was not sufficiently effective against hookworm. Therefore, it was therefore not possible to calculate the level of hookworm egg production, as was done for *O.bifurcum*.
Acknowledgements

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Egg production

References


