Chapter 8

QUANTITATIVE INTERPRETATION OF COPROCULTURES, IN A POPULATION INFECTED WITH *OESOPHAGOSTOMUM BIFURCUM*

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Abstract

Coproculture is used in northern Togo and Ghana in the research on *Oesophagostomum bifurcum*, a common parasite of man in these regions. Prior to a follow-up study to investigate patterns of reinfection in *Oesophagostomum* and hookworm, it was attempted to evaluate the relevance of counting larvae for the assessment of the intensity of infection at the population level.

Of 102 samples, one egg count (Kato-smear) and three coprocultures were carried out. Frequency distributions of counts of larvae of *Oesophagostomum* and of hookworm isolated in three coprocultures, showed log-normality. There was a highly significant correlation between egg counts and the combined number of *Oesophagostomum* and hookworm larvae (Spearman rank correlation test, $r = 0.74$, $p < 0.01$).

It is concluded that the mean larval counts of three coprocultures can be interpreted quantitatively, as this is normally done for egg counts. A quantitative classification of larval counts is proposed.
Introduction

*Oesophagostomum bifurcum* is a common parasite of humans in northern Togo and Ghana [1]. Infection with this nematode is studied using coproculture as the diagnostic method. The use of this rather laborious procedure is necessitated by the almost complete morphologic similarity of the eggs of *Oesophagostomum* and hookworm. In contrast, the infective third-stage larvae of both species, which are prevalent in the same villages and often in the same individuals, can easily be differentiated [2].

Part of the research on *O. bifurcum* focuses on the transmission dynamics, by studying the degree of re-infection after anthelmintic treatment, in different seasons and with different treatment schedules [3]. For that purpose, it is important not only to observe changes in infection rates but also in the intensities of infection. In a small scale study among heavily infected subjects, a significant correlation was observed between the number of larvae cultured and the number of adult worms isolated after treatment [4].

In this paper, infection rates and intensities of *O. bifurcum* were examined in the research population of the above-mentioned follow-up study. Frequency distributions of egg counts, and of *Oesophagostomum*- and hookworm larval counts were evaluated, and the correlation between egg counts and larval counts was analysed. Finally, a classification into categories of light, moderate and heavy infections, based on the numbers larvae found in coprocultures, was established.

**Materials and methods**

In two villages, Lotogou and Dassoute, volunteers were screened for infection with *Oesophagostomum* using one coproculture. Persons found to be infected with *Oesophagostomum*, were asked to participate in the follow-up study, and another stool sample was collected. From each of those samples, one single egg count was performed according to the Kato-Katz method (25 mg). Coprocultures were made from the same specimen using approximately one gram of faeces that was mixed with an equal amount of charcoal [1]. Since it is our experience that for unknown reasons, in some coprocultures the larvae fail to develop properly, coprocultures were performed in triplicate. In each coproculture, the number of *Oesophagostomum*- and hookworm larvae was counted. The arithmetic mean number of larvae of the first two, and of all three coprocultures were calculated. The frequency distributions of the log-transformed egg and larval counts were examined, and the correlation between egg counts and total larval counts was calculated (Spearman rank correlation test).
Table 1. Number of negative results, and the range and median of the positive results (n = 102).

<table>
<thead>
<tr>
<th>Egg counts</th>
<th>Negative (%)</th>
<th>Positive range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17 (16.6%)</td>
<td>0 - 513</td>
<td>34</td>
</tr>
</tbody>
</table>

Larval counts of *Oesophagostomum*

<table>
<thead>
<tr>
<th>Coprocultures</th>
<th>One Coproculture</th>
<th>Two Coprocultures</th>
<th>Three Coprocultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Coproculture</td>
<td>22 (21.5%)</td>
<td>1 - 1013</td>
<td>26.5</td>
</tr>
<tr>
<td>Two Coprocultures</td>
<td>18 (17.6%)</td>
<td>0.5 - 521</td>
<td>24.0</td>
</tr>
<tr>
<td>Three Coprocultures</td>
<td>17 (16.6%)</td>
<td>0.33 - 464</td>
<td>22.87</td>
</tr>
</tbody>
</table>

Larval counts of hookworm

<table>
<thead>
<tr>
<th>Coprocultures</th>
<th>One Coproculture</th>
<th>Two Coprocultures</th>
<th>Three Coprocultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Coproculture</td>
<td>31 (30.3%)</td>
<td>1 - 1800</td>
<td>38.0</td>
</tr>
<tr>
<td>Two Coprocultures</td>
<td>27 (26.4%)</td>
<td>0.5 - 1415</td>
<td>34.0</td>
</tr>
<tr>
<td>Three Coprocultures</td>
<td>25 (24.5%)</td>
<td>0.33 - 1343</td>
<td>38.33</td>
</tr>
</tbody>
</table>

Total larval counts

<table>
<thead>
<tr>
<th>Coprocultures</th>
<th>One Coproculture</th>
<th>Two Coprocultures</th>
<th>Three Coprocultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Coproculture</td>
<td>15 (14.7%)</td>
<td>1 - 1853</td>
<td>76</td>
</tr>
<tr>
<td>Two Coprocultures</td>
<td>13 (12.7%)</td>
<td>0.5 - 1476</td>
<td>63.5</td>
</tr>
<tr>
<td>Three Coprocultures</td>
<td>11 (10.7%)</td>
<td>0.33 - 1388</td>
<td>64.67</td>
</tr>
</tbody>
</table>

Results

In Table 1 the ranges of larval counts and the median larval counts are given along with the efficacy of diagnosis based on larval counts of one, two or three coprocultures, all prepared from the same stool specimen. Of those patients known to harbour *O. bifurcum* the percentage of false negative diagnoses can be reduced from 21.5 to 16.6%, when examining three instead of one coproculture.

The frequency distributions of the egg- and larval counts are shown in the figures 1 (A-D). The bar diagrams are based on the arithmetic mean counts of the three stool cultures for each of the subjects. The results of one and two cultures showed an almost identical distribution, and are not given here.

The distributions of the larval counts of hookworm and *Oesophagostomum* in the positive coprocultures are quite similar and approach log-normality. The distributions of the egg counts, and of the combined counts of larvae of hookworm and
*Oesophagostomum* are more skewed. In figure 2 the correlation between the numbers of eggs and larvae of individual subjects is visualized ($r = 0.74$, $p < 0.001$).

**Discussion**

It has not been the aim of the present communication to give a mathematical analysis of the distribution of parasites and eggs over the infected population. Instead, it was the purpose to establish whether or not larval counts may be used as a semiquantitative parameter in describing the parasites' distributions and the intensities of infection in a given population in an endemic area.

Data obtained from small numbers of treated subjects already indicated that the numbers of larvae cultured reflect the numbers of adult worms harboured by the host [4]. When the frequency distributions of the numbers of *Oesophagostomum* and hookworm larvae are analysed, it can be seen that in the majority of subjects few larvae are found whereas the heavy infections are concentrated in a few persons only. Eighty percent of the larvae is produced by a mere 20% of the (most heavily) infected subjects. Similarly skewed distributions are normally found in the negative binomial distributions of egg counts found in hookworm- and other helminth infections [5]. Quantitative studies on the epidemiology of helminth infections are normally based on logarithmic transformations and geometric means of egg counts [6,7,8]. When the larval counts of the *Oesophagostomum*- or hookworm infected subjects, were log-transformed, the transformed data approached a normal distribution.

The frequency distributions of both the egg counts (counting the eggs of both nematode species together) and the totals of larvae counted, significantly deviated from log-normality. However, the sum of two log-normal distributions with different means and standard deviations is unlikely to produce another log-normal distribution. Moreover, infection with *Oesophagostomum* and hookworm have been shown to be dependent variables [9]. The correlation between egg counts and larval counts should, therefore, be analysed with non-parametric methods. In Figure 2 it is shown that, using Spearman's Rank Correlation test, there is a highly significant correlation between the egg counts, generally assumed to reliably represent the worm load, and the total larval counts.
A. Egg counts (Kato-Katz method, 25 mg)

Number of samples

B. *Oesophagostomum* larval counts.

Figure 1(A-D). Frequency distributions of egg counts (Kato-Katz method, 25 mg), and of larval counts of *Oesophagostomum*, hookworm and total larval counts (arithmetic mean of three coprocultures). The numbers of eggs or larvae were classified into groups as follows:
C. Hookworm larval counts

Number of samples

D. Total larval counts

group 0 (no eggs/larvae found), 1 (1), 2 (2-3), 3 (4-5), 4 (6-9), 5 (10-17), 6 (18-31), 7 (32-56), 8 (57-99), 9 (100-171), 10 (178-316), 11 (317-561), 12 (562-999), 13 (>1000 eggs/larvae found).
Log [larval counts +1]

3,5

3

2,5

2

1,5

1

0,5

0

0

0,5

1

1,5

2

2,5

3

Log [egg counts +1]

Figure 2. Correlation between egg counts (eggs per Kato-smear, 25 mg) and larval counts
(arithmetic mean of three coprocultures) (Spearman rank correlation test, \( r = 0.74; p < 0.001 \)).

The combination of these observations: the significance of the Spearman correlation
test between egg and larval counts, the log-normal distribution of species-specific larval
counts, and the similarity of the distributions of hookworm and *Oesophagostomum*
larvae, would allow the quantitative interpretation of the log-transformed counts of
hookworm and *Oesophagostomum* larvae. On the basis of these observations, larval
counts of *Oesophagostomum* and hookworm were classified into five groups, each
group representing a class-width of \(10^{0.5}\) (table 2).

The results indicate that the prevalence of *Oesophagostomum*-positive cultures increases
from 80.3% (80/102) to 83.3% (85/102) when three cultures are performed instead of
one. For hookworm the increase of the sensitivity by carrying out the coproculture in
triplicate, is of the same order of magnitude. The data presented in table 1 do not
really justify the procedure used hitherto, i.e. to carry out all stool cultures in
triplicate. Yet, occasional differences may be important. In one case, 1013
*Oesophagostomum* larvae were cultured in the first culture, and 29 and 38 in the
subsequent cultures. Moreover, experience suggests that in some months of the year, more cultures tend to fail than in other periods: eggs are found but larvae cannot be cultured. Therefore, in the above-mentioned follow-up study coprocultures were performed in triplicate, and the results were classified into five groups shown in table 2.

Table 2. Proposed classification for mean larval counts of three coprocultures.

<table>
<thead>
<tr>
<th>Number of larvae</th>
<th>'Larval Score'</th>
<th>Number of samples as for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Oesophagostomum</em></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>1 - 9</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>10 - 31</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>32 - 99</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>&gt;100</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>102</td>
</tr>
</tbody>
</table>
References


