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Multiple Stool Examinations for Ova and Parasites and Rate of False-Negative Results

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A mathematical model that shows the relationship between the number of stool examinations for ova and parasites and the rate of false-negative results is described. An analysis of 1,869 patients with three stool examinations revealed various sensitivities for different parasites.

The microscopic demonstration of parasites in fecal samples is not always achieved in every sample, even in heavily infected subjects. Besides possible methodological problems, this difficulty is due to the fact that many cysts or ova are excreted irregularly. In routine diagnosis, one tries to overcome this problem by examining several stool samples produced on different days (1). Therefore, it is recommended that for symptomatic patients, at least three samples be examined for the presence of parasites, especially protozoans. This costly procedure greatly increases sensitivity but has a purely empiric background. Depending on the question being asked (individual diagnosis, epidemiological survey, costs) and the consequences of a false-negative result, the optimal number of stool samples may vary. Using a mathematical model, we looked at the relationship between the number of sodium acetate-acetic acid-formalin (SAF) fixed stools examined and the rate of false-negative results obtained for the most frequently found parasites.

The method is described by Mullen and Prost (2) and was adapted for our purpose. They give two equations for the estimates of α, the prevalence of the population, and p, the sensitivity of an individual test:

$$\alpha = \frac{N - N_0}{N[1 - (1-p)^n]} \text{ and } \alpha = \frac{\Sigma\, k\, N_k}{N\, n\, p}$$

where N is the number of individuals, each sampled n times, and N_k is the number of individuals with k positive samples. Equations describing the variances of these estimates can be found in reference 2. These equations can be manipulated to find the number of samples required so that the proportion of false-negatives becomes smaller than any chosen level R:

$$n > \frac{\ln(R)}{\ln(1-p)}$$

A computer program, written in C, to evaluate these equations can be obtained from one of the authors (J.C.K.).

The equations were applied to the results of the examination of three different stool specimens from 1,869 patients at the Diagnostic Center of the Swiss Tropical Institute. Each specimen was fixed in SAF fixative and concentrated as described by Yang and Scholten (3). The sediment was then microscopically examined for ova and parasites by experienced laboratory technicians, and the number of positive specimens was recorded for each patient. For each parasite, the recorded frequency distribution allowed an estimation of the true prevalence as well as the probability of correctly identifying an infected individual with a single stool examination. The results are compiled in Table 1. It is obvious that *Entamoeba histolytica* has the lowest detection rate, with a probability of only slightly above 60% of correctly identifying an infected individual with a single stool examination. In comparison, *Giardia lamblia* is more easily detected by the SAF method than *E. histolytica*. This also holds true for the merthiolate-iodine-formalin method, although the probabilities for both parasites are about 10% lower (data not presented). Helminth eggs are readily detected. This result may be due either to the fact that they are more easily identified in the microscope or to the fact that they are excreted more regularly than protozoan cysts or trophozoites. The calculations also reveal that the recommendation of examining three different stool specimens will result in a probability of <2% of misclassifying an infected individual, with the nota-

TABLE 1. Frequency distribution of the most common parasites in stool specimens from 1,869 patients[a]

Organism	No. of patients for whom the following no. of samples were found positive:			Total no. of infected patients	Prevalence (%)	Probability (%)[b]	% False-negatives for 3 samples
	1	2	3				
Entamoeba histolytica	51	25	45	121	6.5	61.2	5.8
Giardia lamblia	34	27	72	133	7.1	75.0	1.6
Trichuris trichiura	4	8	36	48	2.6	88.8	0.1
Ascaris lumbricoides	2	2	20	24	1.3	91.6	<0.1
Hookworms	5	5	13	23	1.2	77.4	1.2

[a] Three specimens from each patient were examined.
[b] Probability of identifying an infected individual by examination of a single stool sample (sensitivity of an individual test).

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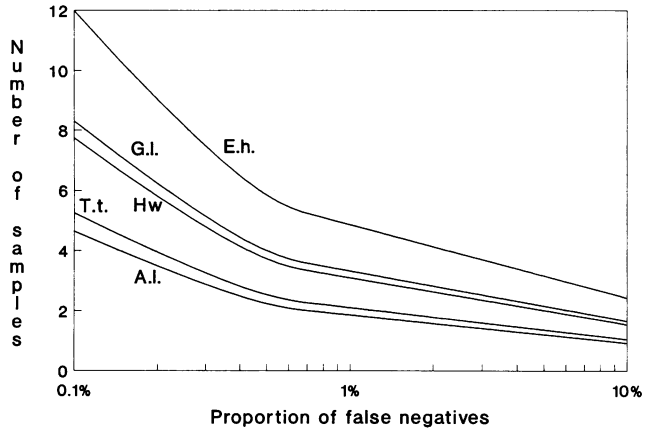

FIG. 1. Relationship between the number of stool samples examined for ova and parasites and the proportion of false-negative results obtained for different parasites. E.h., *E. histolytica*; G.l., *G. lamblia*; Hw, hookworms; T.t., *Trichuris trichiura*; A.l., *Ascaris lumbricoides*.

ble exception of *E. histolytica*. As shown in Fig. 1, more than four specimens are necessary for this parasite to reach the same level of performance. It must be pointed out that the curves shown in Fig. 1 are calculated for the SAF method and therefore should not be applied when different techniques are used, as they may have various sensitivities for the detection of parasites.

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