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The TIF Direct Smear as an Epidemiological Tool

With Special Reference to Counting Helminth Eggs *

F. L. DUNN [1]

Thiomersal–iodine–formalin (TIF) has been used as a faecal preservative in many prevalence surveys of intestinal helminths and protozoa. In helminth surveys, however, estimates of worm burden are no less essential than those of prevalence. Direct-smear and dilution egg-counting techniques, using fresh faeces, have been developed to provide such estimates. This study originated in a field survey that required the use of a preservative. Attempts to estimate worm burden with TIF-preserved faeces led to the assessment of TIF direct-smear (TIF–DS) methods reported here. TIF–DS egg-counting provides reliable statistical estimates of hookworm, Ascaris *and* Trichuris *burden; this is a satisfactory method for estimating worm burden when faeces must be preserved and transported from the field. TIF direct-smears also determine parasite prevalence "efficiently". Some previous studies have cast doubt on the value of TIF concentration (TIFC) and in this study also TIFC proved to be erratic or ineffective in concentrating eggs.*

The studies reported here grew out of an investigation of intestinal parasitism in the aboriginal peoples (Orang Asli) of Malaya (West Malaysia). Surveys were conducted in the field and at a hospital for the aborigines in 1962–64 (Dunn & Bolton, 1963). Because field-preservation of faecal specimens was essential, the thiomersal [2]–iodine–formalin (TIF) method was adopted (originally described by Sapero & Lawless (1953) as the Merthiolate–iodine–formalin (MIF) method). Egg-counting was undertaken on direct smears of TIF-preserved material to estimate worm burdens. Egg-counting from direct smears (of fresh faeces in saline) is an established technique (Beaver, 1950), but attempts to count helminth eggs in smears of TIF-preserved faeces have not previously been reported.

To evaluate the TIF direct-smear (TIF–DS) technique as an epidemiological tool, studies were designed to assess: the variability and reliability of the egg-counts and the effectiveness of TIF–DS examinations in providing a true measure of parasite prevalence in a survey population. These studies focussed on 3 intestinal nematodes: *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm (primarily *Necator americanus*). Reliable and useful data on egg-counts were obtained with TIF smears. The efficiency with which light infections were detected was also sufficiently high for most epidemiological purposes.

MATERIAL AND METHODS

Faecal specimens were collected and preserved in TIF following standard methods (Sapero & Lawless, 1953). All specimens were placed in test-tubes (15 cm by 1.5 cm) containing 2.5 ml of TIF solution. Lugol's iodine (0.15 ml) was first added to each collecting tube with a tuberculin syringe and a long needle. Just before inserting the faecal sample, 2.35 ml of thiomersal–formalin was added to the iodine to form the final TIF mixture. Iodine crystals (1 g), potassium iodide (2 g) and distilled water (20 ml) were used to prepare each 20 ml of Lugol's iodine for stock; this solution was used within 3 weeks of preparation. Glycerol (5 ml), formalin (25 ml), commercial 1 : 1000 tincture of thiomersal (200 ml) and distilled water (250 ml) were used to prepare 480 ml of thiomersal–formalin, sufficient for about 200 TIF specimens.

Faecal samples about the size of a large pea and weighing approximately 200 mg–250 mg were taken

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[1] Associate Professor, Department of Epidemiology and International Health, University of California Medical Center, San Francisco, Calif., USA.

[2] International Non-proprietary Name.

and inserted into the tubes on bamboo splinters. The samples were then thoroughly mixed in the TIF. (In the studies reported here, all specimens were formed or soft/semi-formed. No attempt was made to include liquid specimens in the comparisons because the validity of egg-counts on liquid specimens—by any method—is open to question, and because the method under evaluation was developed for epidemiological survey purposes. Except in times of epidemic diarrhoea and dysentery, when a helminth survey would in any case be deferred, liquid specimens are relatively infrequent in the field. Under hospital conditions liquid specimens may be much more frequent, but the TIF–DS technique is not presented as a clinical tool.)

Two studies are reported. In the first, egg-counts for several types of TIF direct smears were compared with each other and with counts on smears prepared after TIF ether-centrifugation concentration (TIFC) (Blagg et al., 1955). For this study, specimens were collected from 75 Temuan aborigines living near Kuala Lumpur. In the second study TIF–DS nematode egg-counts were compared with saline direct-smear counts for the same faecal samples—40 fresh specimens obtained from hospitalized aborigines.

In an undisturbed, upright tube or vial, the TIF specimen forms 3 well-defined layers within 24 hours of collection (Fig. 1). The upper layer, a clear orange fluid, consists mainly of formalin, thiomersal and water. No surface pellicle forms over this fluid; the surface does not trap eggs or protozoa. The interface, a thin, pale-orange or creamy yellow middle layer, usually 1 mm–2 mm thick, consists of light particulate material. This layer may trap helminth eggs but definitely does not "concentrate" those of *Ascaris*, hookworm, or *Trichuris*. There is some evidence that protozoa may be more numerous in the interface than in the deeper sediment. The third, or lower, layer is more or less homogeneous, consisting of deeper-staining, orange or reddish orange particulate matter. Eggs and protozoa may be found throughout this layer. The base, in this paper, refers to the zone at the base of the lower layer, just above the bottom of the tube (where heavy, gritty particles that interfere with the smooth formation of smears settle).

TIF direct smears were prepared with material removed with a Pasteur pipette from the interface or the base. TIF mixed direct smears were also made, with material drawn from the midpoint of the specimen after thorough re-mixing (Fig. 1). About 0.05 ml of clear fluid was taken into the pipette together with a sample of the interface or base sediment. The mixed suspension contained a similar volume of diluting fluid. In the earliest studies, a crayon mark or a notch filed on the pipette near the tip was used to indicate the length of tubing to be filled, with either clear fluid and sediment or with a mixed suspension. This mark was usually placed 20 cm–25 cm above the tip, the position varying with the diameter of the tubing near the tip of each pipette. Later this was found to be unnecessary; visual judgement alone was sufficient to prepare a series of smears of approximately the same density.

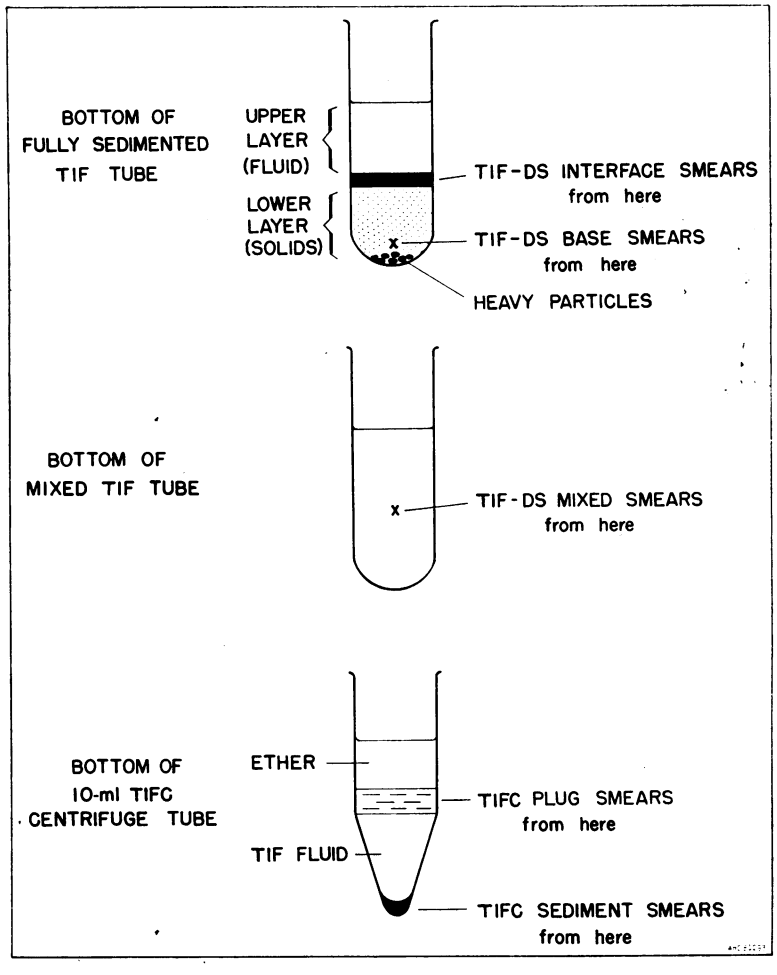
Base and interface smears are "standardized", regardless of the consistency of the faecal sample or of variations in the volume and weight of the sample, because sediment is sampled (by definition) and it is not difficult to pick up approximately the same volume of sediment and diluting fluid with each successive pipette load. The density of the mixed suspension does, however, reflect the initial consistency of the faecal sample as well as any variations in sample size. In pipetting for the mixed smear it is therefore necessary to vary slightly the volumes of pipetted suspensions to produce smears of constant density; again, with a little practice, this presents no difficulties. The "control" in making any of these direct-smears is "a smear that looks right" to the investigator's eye. With practice the investigator can make smears of nearly constant density almost automatically. Routine photoelectric calibration is unnecessary, laborious and time-consuming.

Smears were prepared under 22 mm by 22 mm glass cover-slips, 1 pair of smears per glass slide. Test smears were calibrated, following the procedures of Beaver (1950, 1961). The "standard" TIF direct smear in this study corresponded in density to a 1/300-ml barium sulfate suspension; thus each smear contained approximately 3 mg of formed faecal material. The normal time for preparing 2 smears, making a careful search and counting eggs was 15–20 minutes, depending somewhat on the abundance of eggs. One microscopist can easily examine and count 10–12 specimens in 3–4 hours.

It was found convenient in counting helminth eggs to scan each smear completely, by horizontal traverses, under a total magnification of $\times 70$. To ensure recognition of all protozoa, smears were also re-examined at $\times 200$. All counts were carried out under "blind" conditions. I read and counted all smears in the first study; my assistant and I each read half of the smears in the second study.

FIG. 1

NOMENCLATURE USED IN THIS PAPER IN REFERRING TO FAECAL SPECIMENS PRESERVED IN TIF IN TEST-TUBES, AND TO SPECIMENS AFTER TIFC CENTRIFUGATION AS THEY APPEAR IN THE CENTRIFUGE TUBE



In the first study, eggs of *Trichuris*, *Ascaris*, and hookworms were counted on 6 sets of TIF direct smears, as follows: 2 interface smears (interface I and II), 2 base smears (base I and II), and 2 mixed smears (mixed A and B). All specimens were then concentrated by the TIFC method (Blagg et al., 1955). Smears of TIF-DS density were prepared from the TIFC sediment—the button of sediment at the bottom of the centrifuge tube—and from the plug of detritus at the junction of the ether and TIF fluid layers (Fig. 1). Counts were performed on 75 TIFC sediment smears and 71 TIFC plug smears. Plugs from 4 centrifuge tubes were accidentally discarded.

For the second study, 3-mg direct smears were prepared with fresh faeces and 0.05 ml of diluting saline. A portion of each fresh faecal specimen was also preserved in TIF. Saline and mixed TIF direct smears were then examined and counted for each of the 40 samples.

Two terms used frequently in the description of results require to be defined. "Efficiency", expressed as a percentage, denotes the effectiveness of faecal smear examinations in detecting all infections by a

TABLE 1

INDIVIDUAL AND TOTAL EGG-COUNTS FOR HOOKWORM ON TIF DIRECT SMEARS [a] OF THE INTERFACE, BASE AND MIXED TYPES, AND ON TIFC SEDIMENT AND PLUG SMEARS [a]

Specimen No.	Interface (I)	Base (I)	Interface (II)	Base (II)	Mixed (A)	Mixed (B)	TIFC sediment	TIFC plug
M— 3	7	3	6	7	11	9	1	not done
6	1	—	1	2	—	—	1	4
9	1	3	1	2	2	—	—	—
12	—	2	—	1	—	—	—	1
15	—	—	—	1	—	—	—	—
18	—	1	—	—	1	1	—	1
21	3	—	2	3	1	—	—	—
24	1	4	1	2	2	2	6	1
27	2	1	—	2	1	2	1	2
30	3	1	—	—	1	1	8	1
33	—	2	—	2	2	3	—	7
36	37	32	47	52	44	30	29	21
39	5	24	3	12	11	3	12	—
42	4	—	1	5	3	2	—	1
45	1	1	1	—	—	—	1	—
48	2	6	7	1	6	4	—	8
51	16	24	9	28	8	15	23	16
54	5	1	10	3	3	1	—	7
57	4	—	2	1	3	5	3	2
60	—	—	—	—	—	—	—	—
63	—	—	1	—	1	—	—	—
66	1	—	1	3	3	2	7	—
69	—	—	—	—	—	—	—	—
72	1	1	—	—	—	—	—	—
75	2	1	—	1	—	3	6	—
Total counts for these 25 specimens	96	107	93	128	103	83	98	72
Total count (75 specimens)	261	282	232	257	261	227	234	128
No. of smears positive (75 specimens)	43	43	39	44	44	42	33	32

[a] 3-mg smears.

species of helminth in a survey population. The "survey count" is the mean helminth egg-count per gram of faeces per person infected in a surveyed population.

RESULTS

Hookworm

Egg-counts for 8 sets of TIF–DS and TIFC smears prepared from 75 faecal specimens are summarized in Table 1. To exemplify the individual counts in these studies, hookworm egg-counts for every third specimen are shown; the other counts are totalled for each set of smears.[1]

[1] Full mimeographed tabulations of all individual counts for the 3 species of helminths are available to interested readers from the author.

The total hookworm egg-counts for 6 sets of 75 TIF direct smears ranged from 227 to 282 (mean 253). Counts in base smears were only slightly higher than counts in interface smears. The mean of 244 for the 2 sets of mixed smears is close to the mean for all 6 sets of TIF–DS counts. Hookworm eggs are apparently rather evenly distributed through the TIF sediments. The total counts of a pair of interface and base smears and a pair of mixed smears both approximate the mean count.

The TIFC method performed poorly in comparison with direct smears from TIF specimens; hookworm eggs were not concentrated. The total egg-count for the set of 75 TIFC sediment smears was 234, below the mean of 253 for the 6 sets of TIF direct smears. The total of only 128 for the set of 71

TIFC plug smear counts showed that a surprisingly large number of hookworm eggs may be retained in the plug of detritus.

The 8 sets of TIF–DS and TIFC smears revealed hookworm infections in 65 of the 75 specimens. No single set of smears, however, disclosed more than 44 of these 65 infections. TIFC sediment and plug smears were notably ineffective in identifying hookworm infections. The TIFC sediment smears failed to reveal any infections not identified by any other smear.

The total counts for sets of smears in various pairings are presented in Table 2. A pair of TIF base smears was more effective than a pair of interface smears for recovery of eggs and recognition of very light infections, but was not more effective than combinations of TIF base and interface or TIF mixed smears. No pair of sets of smears revealed more than 56 of 65 hookworm infections in 75 specimens, but 56 detections represent 86% efficiency. At the other extreme the pair of sets of TIFC smears was only 71% efficient in detecting 46 of 65 infections. It cannot be assumed that these 8 sets of smears revealed all possible infections in the sample of 75, but probably not more than 2 or 3 very light infections were missed. Efficiency calculations are shown for the possibility that *all* 75 specimens contained hookworm eggs. Even in this unlikely event the pair of sets of TIF base smears would have achieved 75% efficiency.

The mean total for 6 sets of 75 TIF–DS counts is 253 hookworm eggs in 3-mg smears from 65 infected persons. This is equivalent to 3.9 eggs per 3-mg smear per infected person, or to a survey count of about 1300 eggs per gram of faeces per infected person.

Ascaris

Table 3 presents summary totals for 75 sets of egg-counts. These counts, as for hookworm, compare favourably with each other. The 6 sets of TIF–DS counts ranged from 1606 to 1922, with a mean of 1709 eggs. The difference between the total counts for interface II and base II direct smears suggests an increase in egg concentration toward the base. This difference, however, largely results from counting variations in 3 specimens with high counts ranging between 150 and 400 eggs per smear. The total counts for interface I and base I are similar. The mean total count for the mixed smears (sets A and

TABLE 2

TOTAL HOOKWORM EGG-COUNTS FOR SETS OF TIF DIRECT SMEARS [a] IN VARIOUS PAIRINGS, AND FOR THE PAIR OF SETS OF TIFC SMEARS [a]

Pairs of sets of smears	Interface I and base I	Interface II and base II	Mixed A and mixed B	TIFC sediment and plug	Interface I and interface II	Base I and base II	Combined totals for 8 sets of smears
Total egg-count for 75 specimens	543	489	488	362	493	539	1 882
No. of specimens positive	54	51	52	46	49	56	65
No. of positive specimens missed if maximum positive = 65	11	14	13	19	16	9	0
Efficiency (%) (65 positive)	83	78	80	71	75	86	—
No. of positive specimens missed with theoretical maximum positive = 75	21	24	23	29	26	19	10
Efficiency (%) (75 positive)	72	68	69	61	65	75	87

[a] 3-mg smears.

TABLE 3

TOTAL EGG-COUNTS FOR *ASCARIS* ON SETS OF TIF DIRECT SMEARS [a] OF THE INTERFACE, BASE AND MIXED TYPES, AND ON TIFC SEDIMENT AND PLUG SMEARS [a]

	Interface (I)	Base (I)	Interface (II)	Base (II)	Mixed (A)	Mixed (B)	TIFC sediment	TIFC plug
Total count (75 specimens)	1 675	1 649	1 674	1 922	1 606	1 729	973	1 143
No. of smears positive (75 specimens)	51	54	49	51	49	52	51	40

[a] 3-mg smears.

B) is 1669, close to the mean for all 6 sets of TIF smears. *Ascaris* eggs also appear to be distributed more or less evenly through the sedimented TIF specimen.

TIFC failed to concentrate *Ascaris* eggs. The total egg-count for 75 TIFC sediment smears was little more than half the mean total count for the TIF smears, and the TIFC plug total count was actually higher than that for the TIFC sediment.

Ascaris infections were found in 56 of the 75 specimens by examination of the 6 sets of TIF smears. The TIFC smears failed to uncover any additional infections. One set of TIF base smears (base I) revealed 54 of the maximum 56 infections, but 2 other sets of TIF smears identified only 49 of the 56 infections. The TIFC sediment smears performed nearly as well in revealing infections despite poor performance in concentrating *Ascaris* eggs. Even the TIFC plug revealed 40 of the 56 infections.

Paired smear results are presented in Table 4. In terms of total egg-count the TIFC smear combina-

TABLE 4

TOTAL *ASCARIS* EGG-COUNTS FOR SETS OF TIF DIRECT SMEARS [a] IN VARIOUS PAIRINGS, AND FOR THE PAIR OF SETS OF TIFC SMEARS [a]

Pairs of sets of smears	Interface I and base I	Interface II and base II	Mixed A and mixed B	TIFC sediment and plug	Interface I and interface II	Base I and base II	Combined totals for 8 sets of smears
Total egg-count for 75 specimens	3 324	3 596	3 335	2 116	3 349	3 571	12 371
No. of specimens positive	56	53	54	52	54	56	56
No. of positive specimens missed if maximum positive = 56	0	3	2	4	2	0	0
Efficiency (%) (56 positive)	100	95	96	93	96	100	—
No. of positive specimens missed with theoretical maximum positive = 75	19	22	21	23	21	19	19
Efficiency (%) (75 positive)	75	71	72	69	72	75	75

[a] 3-mg smears.

tion was far inferior to the other smear pairs; nevertheless, TIFC revealed 52 of 56 infections, an efficiency of 93%. All paired sets of TIF smears produced roughly equivalent results, both in total egg-counts and in efficiency in revealing infections. If the maximum number of positive specimens was 56, no TIF–DS pair of sets was less than 95% efficient. Even when the efficiency of various TIF–DS and TIFC smear pairs is calculated against a theoretical maximum of 75 infections in 75 specimens, efficiencies range between 71% and 75%. It is unlikely that more than 1 or 2 *Ascaris* infections actually remained undetected because the egg-counts and worm burdens in subjects who were infected were generally high.

The mean total for 6 sets of 75 TIF–DS counts is 1709 *Ascaris* eggs in 3-mg smears from 56 infected persons. This is equivalent to 30.5 eggs per 3-mg smear per infected person, or to a survey count of 10 160 eggs per gram of faeces per infected person.

Trichuris

Findings from 596 *Trichuris* egg-counts are summarized in Table 5. The total counts reveal several striking differences from the findings for *Ascaris* and hookworm.

(1) *Trichuris* eggs were appreciably concentrated in the basal parts of the sedimented TIF specimen. Total base counts (sets I and II) are considerably higher than total interface counts (sets I and II), and these higher counts reflect excesses of base count over interface count for many individual specimens, not just for a few. The total TIF base counts considerably exceed the mean total count of 1413 eggs for all 6 sets of TIF smears. The mixed smear counts (sets A and B) fall below the mean count, probably reflecting rapid settling of *Trichuris* eggs in the suspension when stirring ceases. Presumably the greater basal concentration of *Trichuris* eggs than of hookworm or *Ascaris* eggs is due to the streamlined shape and higher specific gravity of the *Trichuris* egg.

(2) *Trichuris* eggs were concentrated by TIFC. The total count of 3105 eggs for TIFC sediment smears is more than double the mean TIF–DS total of 1413 eggs. The plug retains relatively few *Trichuris* eggs. The TIFC results are not, however, as satisfactory as they might appear to be at first glance. Individual TIFC counts, compared with counterpart TIFC–DS counts, show that the concentrating power was erratic and unpredictable. Some specimens were well concentrated by TIFC but many others were less concentrated than in TIF smears. This erratic behaviour of TIFC is reflected in the fact that the TIFC sediment smears revealed only 69 of 75 possible infections, although one set of TIF base smears actually revealed 74 of 75.

In Table 6 the paired smear results show even more clearly the disparity in efficiency. The 2 sets of TIFC smears were only 93% efficient in spite of the high total egg-count. The pairs of TIF–DS sets were all extremely efficient (none less than 96%) in identifying *Trichuris* infections.

As for the *Ascaris* infections, high efficiencies reflect relatively high egg-counts. The mean total count of 1413 *Trichuris* eggs in 3-mg TIF smears from 75 infected persons is equivalent to a mean count of 18.8 eggs per 3-mg smear per infected person, and a survey count of 6260 eggs per gram of faeces per infected person.

TIF and saline direct smears

Condensed findings for the second study of 40 fresh faecal specimens are shown in Table 7. Independent egg-counts were closely similar for the same faecal samples in mixed TIF and saline direct smears. Both types of smears were equally efficient

TABLE 5

TOTAL EGG-COUNTS FOR *TRICHURIS* ON SETS OF TIF DIRECT SMEARS [a] OF THE INTERFACE, BASE AND MIXED TYPES, AND ON TIFC SEDIMENT AND PLUG SMEARS [a]

	Interface (I)	Base (I)	Interface (II)	Base (II)	Mixed (A)	Mixed (B)	TIFC sediment	TIFC plug
Total count (75 specimens)	1 302	1 708	1 231	1 617	1 331	1 290	3 105	346
No. of smears positive (75 specimens)	71	74	68	70	69	72	69	43

[a] 3-mg smears

TABLE 6

TOTAL *TRICHURIS* EGG-COUNTS FOR SETS OF TIF DIRECT SMEARS [a] IN VARIOUS PAIRINGS, AND FOR THE PAIR OF SETS OF TIFC SMEARS [a]

Pairs of sets of smears	Interface I and base I	Interface II and base II	Mixed A and mixed B	TIFC sediment and plug	Interface I and interface II	Base I and base II	Combined totals for 8 sets of smears
Total egg-count for 75 specimens	3 010	2 848	2 621	3 451	2 533	3 325	11 930
No. of specimens positive	75	72	72	70	75	74	75
No. of positive specimens missed	0	3	5	5	0	1	0
Efficiency (%) (75 positive)	100	96	96	93	100	99	—

[a] 3-mg smears.

TABLE 7

A COMPARISON OF HELMINTH EGG-COUNTS FOR THE SAME FAECAL SAMPLES ON SALINE DIRECT SMEARS AND ON TIF DIRECT SMEARS OF THE MIXED TYPE

	3-mg TIF-DS (mixed) smear			3-mg saline direct smear		
	Hookworm	*Ascaris*	*Trichuris*	Hookworm	*Ascaris*	*Trichuris*
Total counts for specimens numbered: 1–10	33	968	1 011	37	805	1 049
11–20	13	192	615	16	171	620
21–30	1	204	478	2	155	512
31–40	6	577	93	11	552	95
Total counts for 40 specimens	53	1 941	2 197	66	1 683	2 276
No. of specimens positive	12	23	36	14	23	36

in detecting infections; the totals differed only by 2 infections for hookworm.

DISCUSSION AND CONCLUSIONS

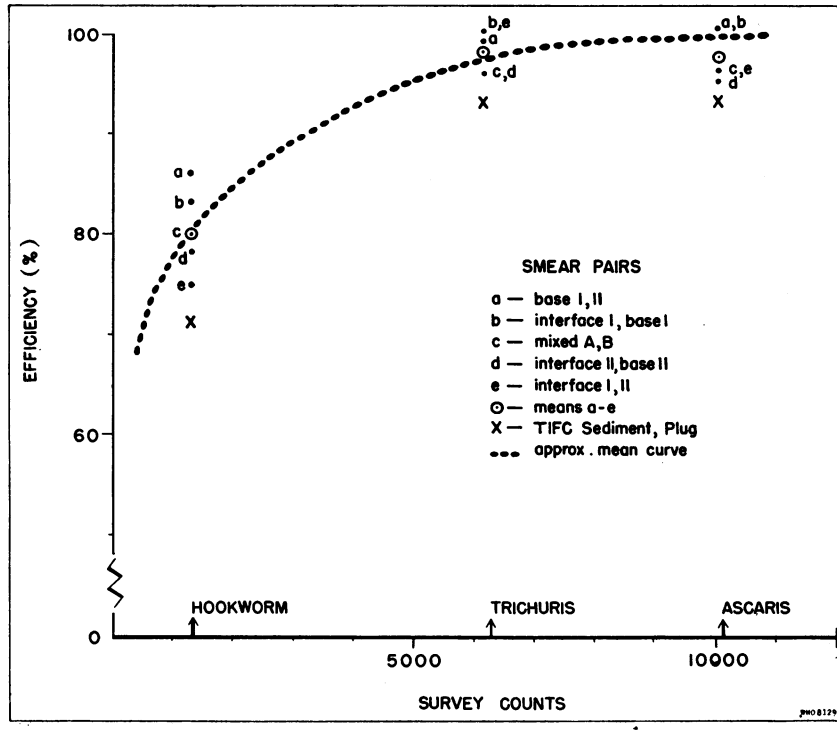
The usefulness of TIF as a preservative for the field collection of faeces is well documented (Kuntz, 1960; Kuntz & Wells, 1962). Surveys utilizing TIF have provided valuable prevalence data for intestinal parasites in many countries. These TIF surveys have not, however, provided estimates of worm burden. Such estimates, which must be based upon egg-counts, are nearly as important as prevalence determinations in evaluating the status of intestinal helminthiases in a population. Since valid egg-counts cannot be made on concentrated specimens, the choice of method narrows to that of Stoll (1962), with its modifications, or to counting of direct smears as advocated by Beaver (1950, 1961). When specimens must be transported from the field, and examination is delayed, the method of Stoll is not feasible; the only available procedure is direct-smear counting of material collected in a preservative such as TIF.

The results of studies reported here on the application of direct-smear egg-counting to TIF-preserved material indicate that TIF–DS counting can provide worm burden estimates at least as reliable as those obtainable by other counting methods. Individual counts were generally similar for each of the visually standardized TIF direct smears prepared from any single faecal sample (Tables 1–6). Table 7 shows that counts on 3-mg mixed TIF–DS and 3-mg saline/fresh faecal direct smears are closely similar. Other workers (Beaver, 1950; Melvin et al., 1956; Maldonado, 1956) have shown that Beaver's standardized saline direct-smear method correlates well with Stoll's dilution method. All these techniques provide relatively crude data that are, however, valuable for epidemiological purposes. The principal objective in egg-counting is to categorize worm burdens in individuals and populations. Except for purposes of calculating survey counts, direct-smear egg-counts should be converted into categories. For example, for 3-mg TIF–DS, I have used the following: 1–2 eggs per smear (+), 3–19 eggs (++), 20–79 eggs (+++), ⩾80 eggs (++++). These egg-count categories correspond, of course, to different intensities of worm burden for each species of helminth. Absolute measurements of worm burden in the individual host are neither feasible nor epidemiologically necessary.

These studies also indicate that the examination of pairs of TIF smears is an efficient means for detecting all or most of the hookworm, *Ascaris* and *Trichuris* infections in a population. The relationship between efficiency and survey count is seen in Fig. 2, showing, for each helminth, the efficiencies calculat-

FIG. 2

EFFICIENCY PERCENTAGES [a] FOR SETS OF PAIRS OF TIF AND TIFC SMEARS PLOTTED AGAINST SURVEY COUNTS FOR HOOKWORM,[b] *TRICHURIS* [c] AND *ASCARIS* [d]



[a] Values taken from Tables 2, 4 and 6. The " mean curve " is based upon the mean efficiency percentages for 5 pairs of sets of TIF smears for each species of helminth.

[b] 1300 eggs per gram of faeces per infected person.

[c] 6260 eggs per gram of faeces per infected person.

[d] 10 160 eggs per gram of faeces per infected person.

ed for each pair of sets of smears (Tables 2, 4, 6). *Ascaris*, with a high survey count, was identified with great efficiency by all TIF–DS pairs and even by TIFC smears (Table 4). If all *Ascaris* infections were identified, the mean TIF–DS efficiency in this study was 97%. *Trichuris* efficiencies (Table 6) were similar to those for *Ascaris*. The mean TIF–DS efficiency was 98% and the survey count was again relatively high. Hookworm efficiencies were lower, but not strikingly so, despite a much lower survey count. The TIF–DS efficiencies, calculated on the assumption that all possible infections were detected, range from 75% to 86% with a mean of 80% (Table 2). Even with a survey count of only 1300 eggs per gram, the efficiency of direct-smear egg-counting stays relatively high.

Inspection of Fig. 2 suggests that efficiency must fall off very rapidly for survey counts ranging below 500 eggs per gram. This must be true for any egg-counting procedure. When survey counts are very low, special measures are needed to detect every single light infection. These may include examination of multiple direct smears, use of concentration techniques, or use of Kato's Cellophane thick-smear technique, e.g., as employed by Komiya & Kobayashi (1966). These special techniques may be necessary in helminth eradication projects but for ordinary pilot surveys and most epidemiological purposes they are not needed.

If the mean efficiencies for the 3 survey count levels in Fig. 2 are connected, a tentative "mean curve" can be drawn, a curve that "breaks" far to the left. (It would be more valid to construct such a curve with efficiency data for a series of different survey count levels for a single species of helminth.) If "survey count-efficiency curves" of this type prove to be valid they could be used to estimate efficiency, and thus to provide a rough idea of the actual number of light infections that have been missed in a given survey. For example, a survey of 100 persons might reveal 45 hookworm infections by TIF–DS egg-counting, with a survey count (easily calculated from the individual counts) of 5000 hookworm eggs per gram. By use of the curve in Fig. 2 this survey can be estimated to have been about 95% effective in identifying all infections; probably only 2 or 3 very light infections were missed. (Actually, for this example, it would be preferable to use a more refined curve based on efficiencies for a series of studies of hookworm at different survey count levels.) Estimations of this kind will probably turn out to be valid, at least for the common "soil-transmitted" intestinal helminths whose egg-count distribution curves are similar, tending to approximate to normal curves when plotted on logarithmic scales (Scott, 1942).

For greatest efficiency and reliability in TIF–DS egg-counting 2 mixed smears, or an interface and base pair, may be used. TIF interface smears tend to underconcentrate eggs and are the least efficient in revealing very light infections. TIF base smears are most efficient in detecting light infections, but their tendency to concentrate eggs is undesirable. TIF mixed smears are nearly as efficient as base smears, and counts on these are not distorted by concentration. Two TIF mixed smears are recommended when a survey is limited to determination of helminth prevalence and burden. An interface and base pair of TIF smears is recommended when a survey is concerned with both helminths and protozoa. (Quantitative studies of protozoa were not undertaken, but trophozoites and cysts were clearly most abundant in the interface smears.)

Two smears rather than only 1 are recommended to increase the efficiency in detection of light infections. When a helminth survey count is low (e.g., hookworm in this study) the use of 2 smears increases the efficiency substantially (cf., hookworm positive totals for single sets of smears and pairs of sets of smears in Tables 1 and 2). If laboratory time is short, however, a single set of smears may be examined initially. Rough calculations of survey counts for each helminth species detected in this series of examinations will then indicate whether or not a second set of smears should also be examined.

The comparative investigation of TIFC has demonstrated that this method (at least in its orinal form, which is still in common use) is not so effective as TIF–DS examination for detecting all possible infections. TIFC failed to concentrate *Ascaris* or hookworm eggs; it did concentrate *Trichuris* eggs, but the results were erratic. The TIFC procedures were carried out as described by Blagg et al. (1955) in their original paper. Although the mesh of the gauze used in filtration may have been slightly different, inspection of individual *Trichuris* counts has shown that gauze filtration has no general and consistent egg-trapping effects. The plug of detritus is also responsible for loss of concentrating power; surprisingly high egg-counts, particularly for *Ascaris*, were made on many plug smears.

Kuntz (1960) and Kuntz & Wells (1962) have presented data that agree with these TIFC observations. Their comparisons of TIF and TIFC preva-

lence data suggested that TIFC did not concentrate hookworm eggs, concentrated *Ascaris* eggs slightly, and concentrated *Trichuris* eggs moderately well. Their comparisons even favoured TIFC because the TIFC prevalence values were compared with values obtained by examination of interface smears—shown in the present study to be the least efficient TIF direct smears for detecting helminth eggs.

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RÉSUMÉ

L'auteur expose les résultats obtenus par l'examen d'étalements directs de fèces conservées dans une solution de thiomersal-iode-formol (TIF) pour la recherche des œufs d'helminthes. Cette méthode de conservation des échantillons a été employée au cours de nombreuses enquêtes sur la prévalence des parasites intestinaux, mais elle n'a pas encore été appliquée aux recherches destinées à évaluer l'importance de la charge parasitaire dans une collectivité.

L'étalement direct de spécimens conservés en TIF convient parfaitement aux numérations des œufs de parasites. Au cours de l'étude de trois helminthiases (infections à *Ascaris lumbricoides*, *Trichuris trichiura* et *Necator americanus)*, on a pu apprécier la charge en vers avec des résultats aussi bons que ceux que donne l'examen d'échantillons de selles fraîches par étalement direct ou après dilution. Le procédé présente d'indéniables avantages lorsque les conditions de l'enquête imposent un certain délai entre le prélèvement et l'examen au laboratoire.

L'examen d'étalements directs d'échantillons conservés en TIF est aussi un moyen efficace d'évaluer la prévalence d'une helminthiase. Le rendement de la méthode est d'autant meilleur que le taux d'infection au sein d'une collectivité est élevé. Les données recueillies peuvent servir à établir une courbe des variations du rendement en fonction du taux d'infection qui permettra peut-être de calculer par extrapolation le nombre des infections légères qui sont passées inaperçues.

Une analyse comparative des résultats obtenus par la méthode classique de la concentration des échantillons conservés en TIF montre que le procédé ne permet pas un enrichissement suffisant en ce qui concerne les œufs d'*Ascaris* ou de *Necator*; avec *Trichuris*, les résultats sont inconstants.

La numération des œufs sur étalements directs de fèces fraîches ou conservées en TIF est une technique facile qui offre des garanties suffisantes d'efficacité et d'exactitude dans la plupart des cas. Les méthodes plus laborieuses et plus compliquées n'apportent que des avantages négligeables. Sans concentration des échantillons, il est évidemment impossible de déceler toutes les infections légères, mais ce genre de données n'est habituellement pas indispensable dans une enquête épidémiologique. Si le but recherché est l'éradication complète d'une helminthiase, les techniques de concentration pourront faciliter le dépistage d'un maximum de cas mais elles gêneront les estimations de la charge parasitaire qui ont au moins autant d'importance que les données relatives à la prévalence.

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