

## EVALUATION OF BORDA & PELLEGRINO'S TECHNIQUE IN THE QUANTITATIVE DIAGNOSIS OF HOOKWORM INFECTIONS

C. Edgardo BORDA (1), María J. F. REA (2) and Enrique P. SANCHEZ (3)

### SUMMARY

One hundred and sixty stool specimens of patients infected with hookworms and other helminths were comparatively examined with BORDA & PELLEGRINO's direct thick smear quantitative technique, and STOLL & HAUSHEER dilution. It was observed that in the former, the use of polyethylene glycol, formaline and saturated saline clears the feces and also fixes and preserves the eggs of hookworms, *Ascaris lumbricoides* and *Trichuris trichiura*. These eggs were kept unaltered for at least 256 days (total period of observation), at room temperature, contrarily to what happened with STOLL & HAUSHEER's technique. Besides, the material can be examined in less time, due to the simplicity of the technique. The egg counts per gram of feces (EPG) for the estimation of the degree of infection by hookworm were reproduced in 31.25% of the cases, while in 68.75% they did not coincide. These results have shown that both techniques have an equivalent sensibility in the quantitative appreciation of hookworm infections. From this study, it is concluded that BORDA & PELLEGRINO's is a reliable technique for the quantitative diagnosis of hookworm and other helminth infections.

### INTRODUCTION

In a previous paper it has been shown that employing a solution of polyethylene glycol, formaline and saturated saline, in the proportion of 1:1:2, the eggs of *Schistosoma mansoni* passed out in the feces of monkeys experimentally infected were easily preserved and visible by direct examination (BORDA & PELLEGRINO<sup>1</sup>). Likewise it was determined that the hookworm eggs were preserved for a month, contrarily to what happened with the KATO & MIURA's<sup>6</sup> thick-smear technique, in which they collapse and disappear a short time after the thick-smear is prepared (MARTIN & BEAVER<sup>7</sup>, ZAMAN & CHEONG<sup>12</sup>; JUECO<sup>5</sup>). However it was necessary to evaluate the advantages and

limitations of the former technique in the quantitative examinations of feces of individual cases and in epidemiological studies of that helminthiasis.

The object of this report has been: 1) to determine how long the eggs of hookworms, *Ascaris lumbricoides* and *Trichuris trichiura* can be preserved and identifiable on a smear prepared with the solution of polyethylene glycol, formaline and saturated saline, and 2) to evaluate statistically the technique named, determining the sensibility and reproductibility of its results in the counts of hookworm eggs compared to a dilution method of known efficacy.

- (1) Centro Nacional de Parasitología, Facultad de Medicina, Universidad Nacional del Nordeste, Corrientes, Argentina
- (2) Fellow of the "Comisión Permanente de Investigaciones Científicas y Técnicas de la U.N.N.E."
- (3) Facultad de Ciencias Económicas de la U.N.N.E.  
Address for reprints: Centro Nacional de Parasitología, Santa Fé N.º 1432, Corrientes, Argentina.

#### MATERIAL AND METHODS

The study was done on 160 stool samples of hospital and ambulatory patients infected with hookworms, which were processed in a period of 24 hours.

The determination of the sensibility of the results reached with BORDA & PELLEGRINO's technique, was compared to the results obtained with STOLL & HAUSHEER's method. Egg counts were made in triplicate, corresponding each to that obtained in 30 mg of feces in the first case and 10 mg in the second, using slides of 26 x 75 mm and polyethylene coverslips of 24 x 32 mm. As advised by STOLL<sup>9,10</sup> and more precisely described by SCOTT & HEADLEE<sup>8</sup>, the feces were classified according to their consistency in one of the following four categories: F (formed), SF (soft formed), M (mushy), MD (mushy diarrhetic); then they were reduced to base F, for this purpose the counts from SF specimens were multiplied by one and half, from M specimens by two, and from MD by three.

Otherwise, in order to check the fixing and preserving properties of the solution of polyethylene glycol, formaline and saturated

saline, 40 of the 160 specimens examined with the direct smear were kept in slides at room temperature and examined one, two, four, eight, 16, 32, 64, 128 and 256 days after. Besides hookworm eggs some of these preparations had also eggs of *A. lumbricoides* and *T. trichiura*.

For comparison purposes, the analysis of correlation and regression was applied to determine if the numbers of EPG found in the former were reproduced in the latter, if one was superior or inferior to the other. In Table I, the quantity of eggs found by each method is indicated from 0 to 45.00 or more EPG.

#### RESULTS

In a sample of 40 smears chosen at random and kept at room temperature, it has been found that eggs of nematodes can be preserved for a period of 256 days. The hookworm eggs exhibited the shell and the internal structure well preserved, but in some cases the outer edge of the shell folded up towards the inside covering part of the blastomeres, as shown in Fig. 1. A. Eggs of

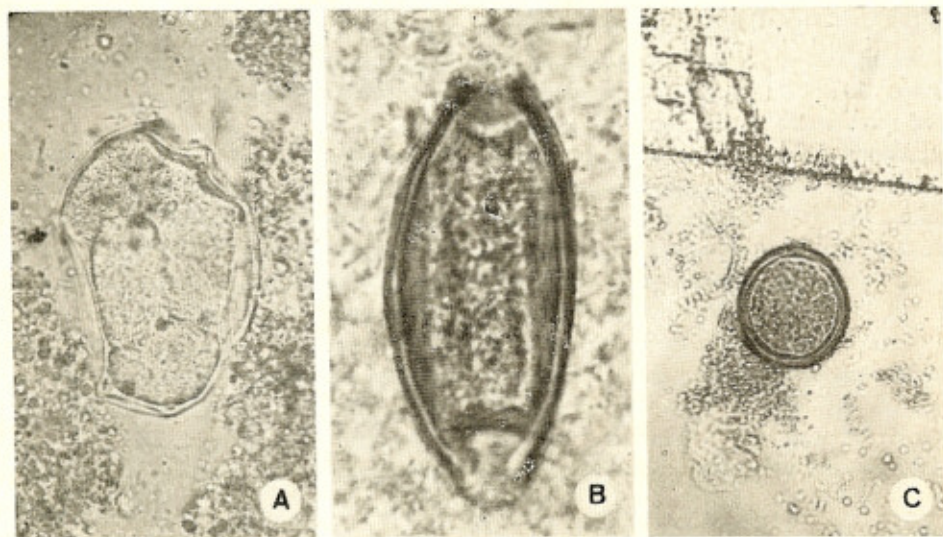


Fig. 1 — Photomicrographs of helminth eggs from a slide prepared 256 days before, with the preservative-clearing solution of polyethylene glycol, formaline and saturated saline. A) hookworm (125 X). B) *Trichuris trichiura* (125 X). C) *Ascaris lumbricoides* (40 X)

*T. trichiura* and *A. lumbricoides* remained equally unchanged during the period of observation (Fig. 1, B and C). It was verified that as time passes sodium chloride crystals of various sizes are formed in some parts of the smear, but, usually in the borders where the surfaces are more exposed to drying.

The values presented in Table I were obtained from the statistic evaluation of the accounts made with both techniques. It is observed from the results that in 50 cases (31.25% from the total), situated in the diagonal, both techniques coincide in their results, while in the remaining (68.75% from the total) situated outside the diagonal, the values obtained were not coincident. From the analysis of each one of the lines the following is observed: 1) From the 40 cases registered by BORDA & PELLEGRINO's technique between 101-800 EPG, compared with STOLL & HAUSHEER's, in 20 (50.00%) the results reproduced in 18 (45.00%) and in two cases (5.00%) showed greater or lesser degree of infections; 2) From the 40 cases registered by BORDA & PELLEGRINO's method between 801-3.000 EPG, nine cases (21.40%) were inside this same interval by STOLL & HAUSHEER's method, while 23 (54.80%) and 10 cases (23.80%) were superior and inferior respectively; 3) From the 19 cases noted by BORDA & PELLEGRINO's method between 3.001-7.000 EPG, only five cases (26.30%) coincided with the result obtained by STOLL & HAUSHEER's method, whereas four (21.30%) and 10 cases (52.60%) denoted greater and smaller elimination of eggs; 4) From the 27 cases situated by BORDA & PELLEGRINO's technique between 7.001-14.000 EPG, only in five cases (29.60%) the accounts were coincident with those of STOLL & HAUSHEER, while, in 10 (37.10%) and in nine cases (33.30%) they were situated between higher and lower ranges; 5) From the 11 cases registered by BORDA & PELLEGRINO's technique between 14.001-22.000 EPG none of the accounts were reproduced by the STOLL & HAUSHEER's method, four cases (36.40%) were over the diagonal and the remaining were under it (63.60%).

With regard to the other intervals, they have not been analyzed because they represent

scarcely 13.00% of the total number of observations.

Diagram I was made with the horizontal and vertical totals of Table I in which were also traced the regression lines obtained of Y in X, and of X in Y. The determination and correlation coefficients were obtained for both regression lines and in both cases the results were 0.82 and 0.91, respectively.

#### DISCUSSION

BORDA & PELLEGRINO's direct thick-smear technique, has shown in the present experiment, to be suitable when it is used to give an estimation of the intensity of the hookworm infection. Besides, in previous studies of experimental infections with *S. mansoni* in laboratory animals (BORDA & PELLEGRINO<sup>2</sup>; BORDA, PELLEGRINO & MESCHESI<sup>4</sup>; BORDA<sup>3</sup>), the small specimens taken directly from the fecal mass served equally for the detection, as well as for the quantitative assessment of the infection through the eggs passed out in the feces.

The experiments beginning with the collection, sieving, extension, weighing and mixing of the fecal specimen with the preserver on the slide, followed by the application of the coverslip is comparatively simpler than STOLL & HAUSHEER's technique and it is carried out in less time. Excluding the trophozoites and cysts of intestinal protozoa, because it is unknown whether they can be discovered and preserved, both, the helminths eggs and the hookworm and *S. stercoralis* larvae can be identified after the preparation has been left to rest for near three minutes, to allow the sedimentation and the clarification of the fecal residues under the polyethylene glycol action. The search can be made immediately or days or months afterwards without any structural alterations in the *S. mansoni*, *A. lumbricoides*, *T. trichiura* eggs, the same as in *S. stercoralis* and hookworm rhabditoid larvae. An infolding of the shell border of hookworm egg occurs, covering, sometimes, part of the blastomeres, as seen in Fig. 1, A, which is, probably, due to liquid currents produced between slide and coverslip during the partial drying of the preparation. In spite of this, the eggs are

TABLE I  
Sensitivity and reproductivity of BORDA & PELLEGRINO's thick smear technique, as compared with that of STOLL & HAUSHEER's dilution in the eggs-counts of hookworm, ranged between 0 and 45,000 or more EPG on specimens from 160 persons

(*) S & H		(***) EPG											TOTAL					
		0	1-100	101-800	801-3000	3001-7000	7001-14000	14001-22000	22001-30000	30001-45000	45001 and more							
(**) B & P	0	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	
	1-100	—	1	3	—	—	—	—	—	—	—	—	—	—	—	—	4	
	101-800	1	1	20	17	1	—	—	—	—	—	—	—	—	—	—	40	
	801-3000	—	1	9	9	18	5	—	—	—	—	—	—	—	—	—	42	
	3001-7000	—	—	4	6	5	2	1	—	—	—	—	—	—	—	—	19	
	7001-14000	—	—	—	4	5	8	4	1	—	—	—	—	—	—	—	27	
	14001-22000	—	—	—	—	3	4	—	—	—	—	—	—	—	—	—	11	
	22001-30000	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	6	
	30001-45000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	
	45001 and more	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	3	5
	TOTAL	3	3	36	37	32	20	5	3	8	13	160						

(\*) STOLL & HAUSHEER  
(\*\*) BORDA & PELLEGRINO  
(\*\*\*) Eggs per gram of feces

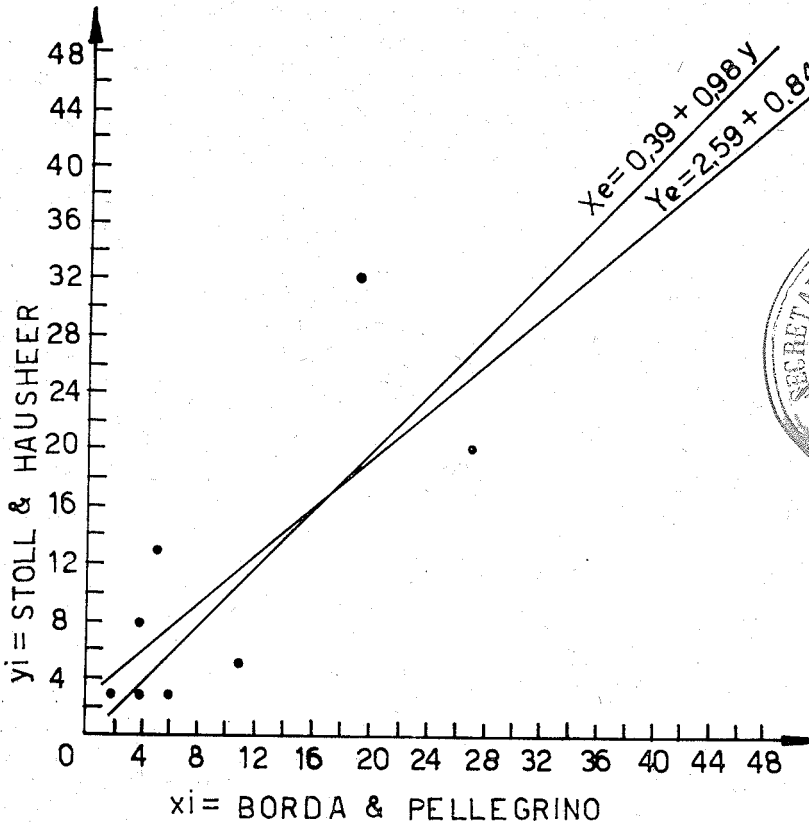
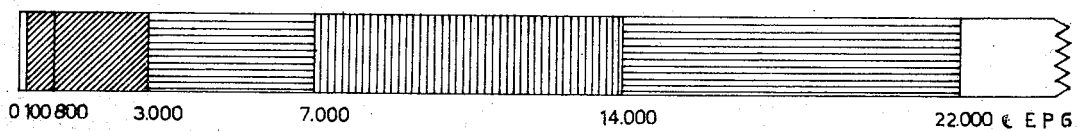


Diagram I — Regression lines obtained between methods

- INTERVALS NOT CONSIDERED.
- INTERVALS IN WHICH STOLL & HAUSHEER'S METHOD SHOWED GREATER OR THE SAME SENSIBILITY AS BORDA & PELLEGRINO'S METHOD.
- INTERVALS IN WHICH BORDA & PELLEGRINO'S METHOD SHOWED GREATER OR THE SAME SENSIBILITY AS STOLL & HAUSHEER'S METHOD.
- INTERVALS IN WHICH BOTH METHODS SHOWED THE SAME SENSIBILITY.



Graphic I — Graphical comparison of the results obtained for both methods according to intervals considered



easily recognized. Moreover, it is also useful for the detection of cestodes (*Tenia saginata*, *Hymenolepis nana*).

As one of the constituents of the preserver is a saturated sodium chloride solution, the desiccation produces the crystallization of that salt, which is increased by the heat and ageing of the preparation. The crystals, of different sizes, sometimes occupy the larger part of the microscopic field interfering with the observation without, however, preventing the recognition of the eggs, as shown in Fig. 1, C, a microphotograph in phase contrast of an *A. lumbricoides* egg next to a crystal, obtained 64 days after preparation of the smear. The number of such crystals diminishes when the supernatant of the preservative saturated salt solution is carefully removed.

Very thick smears seriously delay the examination, chiefly when the feces are very pigmented, but this can be avoided through the use of a larger coverslip (24 x 40 mm). This coverslip allows thinner preparations because the smear diameter is augmented and make the counting easy.

Besides the relative limitations inherent to the technique above mentioned, another problem is represented by the polymer polyetylenoglycol 300 and 105 mesh stainless-steel bolting cloth, recommended by MARTIN & BEAVER<sup>7</sup>, and which may be not available in some localities, mainly in rural areas.

From the statistical evaluation of both techniques (Table I and Graphic I) the following was deduced: 1) In the counts ranged between 101-800 and 801-3.000 EPG of feces, STOLL & HAUSHEER's technique showed a greater or equal sensitivity in 65.00% and 76.00% of the cases, respectively; 2) In the counts that ranged from 3.001-7.000 EPG, BORDA & PELLEGRINO's method was more sensitive in 50.00% of the cases, increasing to 75.00% if the counts reproduced in both techniques are included; 3) Between 7.001-14.000 EPG the percentage difference in both techniques, was very small, in a third of the cases (33.33%) the same sensitivity was observed, whereas in the remaining two thirds, one third corres-

ponded to each method; 4) When the number was between 14.001-22.000 EPG, BORDA & PELLEGRINO's technique showed greater sensitivity in more than 50.00% of the cases; 5) With regard to the intervals situated below 101 and above 22.000 EPG, it can be considered that the number of cases observed was not sufficiently significant to justify statistical conclusions.

The coefficients of determination (0.82) and correlation (0.91) obtained through the regression line, though they can be considered high, from the analysis of the Graphic I, and also from the data previously analyzed, it cannot be accepted for this type of problem in which it is considered that the coefficient of correlation to be acceptable it must not be less than 0.95.

The results of the present study demonstrate that BORDA & PELLEGRINO's technique is efficient both in the diagnostic as well as the quantitative estimation of hookworm infection and other helminthiasis, its sensibility being equivalent to that of STOLL & HAUSHEER's technique and offering the advantage of a simpler execution and durability of the preparation. This last property makes it particularly useful for epidemiological investigation.

#### RESUMEN

*Evaluación de la técnica de BORDA & PELLEGRINO en el diagnóstico cuantitativo de la infección por anquilostomas*

Ciento sesenta muestras fecales de pacientes infectados por anquilostomas y otros helmintos fueron examinadas comparativamente por las técnicas cuantitativas del frotis directo espeso de BORDA & PELLEGRINO y la de dilución de STOLL & HAUSHEER. Se ha observado que la utilización, en la primera, de la solución de polietilenoglicol, formol y salina saturada, clarifica las heces, y además fija y conserva los huevos de los anquilostomas, *Ascaris lumbricoides* y *Trichuris trichiura*. Estos permanecieron inalterados por lo menos 256 días (período total de observación) a temperatura ambiente, al contrario de lo que ocurre con la técnica de STOLL & HAUSHEER. Por otra parte, el

material puede ser examinado en menos tiempo debido a la sencillez del método.

Los contajes de huevos por gramo de heces, para la estimación de la infección por anquilostomas fueron reproducibles en el 31,25% de los casos, mientras que en 68,75% no fueron coincidentes. Estos resultados han demostrado que ambas técnicas poseen una sensibilidad equivalente en la apreciación cuantitativa de la infección por anquilostomas.

Del presente estudio se deduce que la técnica de BORDA & PELLEGRINO es eficaz para el diagnóstico cuantitativo de la infección por anquilostomas y otros helmintos.

#### REFERENCES

1. BORDA, C. E. & PELLEGRINO, J. — An improved stool thick smear technique for quantitative diagnosis of *Schistosoma mansoni* infection. *Rev. Inst. Med. trop. São Paulo* 13:71-75, 1971.
2. BORDA, C. E. & PELLEGRINO, J. — Observaciones sobre la infección experimental de *Didelphis azarae* con *Schistosoma mansoni*. *Rev. Inst. Med. trop. São Paulo* 13:377-382, 1971.
3. BORDA, C. E. — *Infeção natural e experimental de alguns roedores pelo Schistosoma mansoni*, Sambon 1907. [Tese]. Belo Horizonte, Minas Gerais, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 43 pp., 1972.
4. BORDA, C. E.; PELLEGRINO, J. & MESCHESI, H.D. — Eliminación de huevos de *Schistosoma mansoni* por *Cebus apella macrocephalus* infectados experimentalmente. *Rev. Inst. Med. trop. São Paulo* 14:91-96, 1972.
5. JUECO, N. L. — Comparison of Kato's thick smear technic and brine flotation technic in the detection of common helminthic infections. *Acta Médica Philippina* 5:148-151, 1969.
6. KATO, K. & MIURA, M. — Comparative examinations. *Jap. J. Parasit.* 3:35, 1954.
7. MARTIN, L. K. & BEAVER, P. C. — Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections. *Amer. J. Trop. Med. Hyg.* 17:382-391, 1968.
8. SCOTT, J. A. & HEADLEE, W. H. — Studies in Egypt on the correction of helminth egg count data for the size and consistency of stool. *Amer. J. Hyg.* 27:176-195, 1938.
9. STOLL, N. R. — Investigations on the control of hookworm disease. XV — An effective method of counting hookworm eggs in feces. *Amer. J. Hyg.* 3:59-70, 1923.
10. STOLL, N. R. — Investigations on the control of hookworm disease. XXXIII — The significance of egg count data in *Necator americanus* infections. *Amer. J. Hyg.* 4: 466-500, 1924.
11. STOLL, N. R. & HAUSHEER, W. C. — Concerning two options in dilution egg counting small drop and displacement. *Amer. J. Hyg.* 6:134-145, 1926.
12. ZAMAN, V. & CHEONG, C. H. — A comparison of Kato's thick smear technique with zinc sulfate flotation method, for the detection of helminth ova in faeces. *Trans. Roy. Soc. Med. & Hyg.* 61:751, 1967.

Recebido para publicação em 5/5/1975.