

PARASITOLOGICAL DIAGNOSIS OF SCHISTOSOMIASIS MANSONI: FECAL EXAMINATION AND RECTAL BIOPSY

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Even with all progress in the search of sensitive and specific methods for the immunological diagnosis of schistosomiasis, the microscopic detection of eggs of the parasite in the stool still remains the most widely used tool for the actual diagnosis of active infection.

Among the coproscopic methods, Kato's technic modified by Katz et al (Kato/Katz) has the advantages of higher sensitivity, the possibility of egg quantification, its low operational cost and its feasibility in areas with minimal infra-structure.

The oogram of the rectal mucosa is valuable in initial clinical trials of schistosomicides, when it is needed to observe egg morphology in tissue. It could be an alternative method for individual diagnosis, being more sensitive than a single stool exam in low intensity infection. However, the increased sensitivity of a higher number of fecal exams makes that invasive procedure unnecessary.

In the assessment of cure of schistosomiasis, Kato/Katz method (three fecal samples in one, three and six months after treatment) and the rectal biopsy four months after treatment, are equally reliable.

Key words: *Schistosoma mansoni* – fecal examination – rectal biopsy

Currently, the conclusive diagnosis of *Schistosoma mansoni* is most reliably achieved by the direct demonstration of the parasite in one of its developmental forms in the tissues or excretions of the host. Fecal examination as well as biopsy of the rectal mucosa are the parasitological methods used for the diagnosis and evaluation of cure of schistosomiasis mansoni.

The present discussion reviews the most frequently used techniques of the parasitological diagnosis of schistosomiasis and the role of the Kato/Katz method in epidemiological studies of the disease.

FECAL EXAMINATION

Schistosoma mansoni eggs can be detected in the feces by both qualitative and quantitative methods. The selection of appropriate method depends upon the purpose of the diagnosis.

Qualitative methods include: (a) *Centrifugation* – Described by Telleman (1908) this aims to precipitate the heavy eggs through liquid of low density using the force generated by centrifugation. A number of authors have introduced modifications to reduce the quantity of debris in the pellet, to clear the solution obtained and to minimize costs (Faust et al., 1946; Ritchie, 1948; Saper & Lawless, 1953; Blagg et al., 1955; Coutinho, 1956). Even so, this group of methods have a low sensitivity for the diagnosis of schistosomiasis; (b) *Hatching of miracidia* (Fulleborn, 1921) – The technique consists of stimulating the hatching of miracidia present in the fecal specimen by means of appropriate illumination, temperature and osmolarity. Because of their phototropism, the larvae collect in the neck of the flask used where they can be observed with the naked eye or with a lens. The test has been thoroughly analyzed in schistosomiasis mansoni (Zicker et al., 1977; Siqueira et al., 1977; Chieffi et al., 1978). Since it is a sensitive method capable of demonstrating the viability of the miracidium, it is used in the evaluation of new schistosomicides (WHO, 1985); (c)

Spontaneous sedimentation of feces – The technique described by Lutz (1919) and standardized by Hoffman et al. (1934), involves the filtration of homogenized feces through a metal sieve to remove larger fecal residues and to allow the remainder to sediment spontaneously. A sample of the sedimented material is then examined between a slide and cover slip. Modifications in the composition of the liquid, time and number of sedimentations and the substitution of the metal sieve for surgical gauze have been proposed with the aim of producing a clearer liquid to be examined and eliminating the necessity of repeated washing of the sieve (Faust & Meleney, 1924; Martins, 1949). A high level of sensitivity for the diagnosis schistosomiasis mansoni as well as various helminthic and protozoal infections is achieved with this method (Chaves et al., 1979; Rabello, 1990).

Qualitative methods are not recommended for epidemiological studies although they are useful for individual diagnosis, because evaluation based on prevalence does not always reflect the true impact of control programs (WHO, 1985).

In order to estimate the parasite worm burden the number of *S. mansoni* eggs in fecal sample is determined. The estimation of the number of eggs in the feces can be reliably calculated by means of techniques of concentration and smear preparation due to the uniform distribution of *S. mansoni* eggs in the feces (Martin, 1965; Martin & Beaves, 1968).

Among the quantitative methods of stool examination are those of:

Stoll (Stoll & Hausheer, 1926) – A standard flask, known as Stoll flask, containing a NaOH solution, is filled with a known volume of feces. The solution is shaken strongly with glass beads and, after 12 to 24 h, a microscopical examination is undertaken.

Bell (1963) – Formalin fixed and homogenized fecal material eliminated over 24 h is examined. The total sample is force filtered through screens with pores of different dimensions, using a complex apparatus coupled to a vacuum pump. The product of the filtrations is collected on filter paper which is then dried in an incubator, stained and examined by microscopy.

Simões Barbosa (Barbosa, 1965) – This method is essentially the same as the method

of spontaneous sedimentation of feces aforementioned except that a known volume of the sediment is examined and the eggs observed are counted.

Kato/Katz (Kato & Miura, 1954; Katz et al., 1972) – The eggs of *S. mansoni* are concentrated by straining the feces through a screen that removes the larger pieces of debris. The material obtained is then covered by a cellophane cover slip, soaked in a solution of glycerine and malachite green that permits dehydration. Thus, a dry, clear preparation is ready to be examined after 30 to 60 min. Martin & Beaver (1968) introduced the quantification of eggs to the method of Kato by the preparation of a known quantity of feces and the counting of all the eggs observed on the slide. In this way the *S. mansoni* eggs are concentrated by between 103 and 109% (Katz et al., 1970; Teesdale & Amin, 1976). The advantages of this quantitative technique are the simplicity of collection and execution, low cost and the possibility that the slide can be kept at room temperature for several months after preparation without affecting the results. Katz et al. (1972) described a modification of the technique of Kato (the Kato/Katz technique) which consists of substituting the weighing of the feces on an analytical balance by the filling of a hole of known dimensions in a card. Other modifications were suggested by Peters et al. (1980) who described the “quick-Kato” in which smaller amounts of feces are employed allowing the slide to be examined 20 min after preparation.

The quantitative methods of Kato, Bell and Stoll are equivalent in terms of sensitivity (Martin & Beaver, 1968; Coura & Conceição, 1974; Mello et al., 1977). The method of Bell is the most sensitive for the diagnosis of individuals with a low egg output, while the method of Kato/Katz exhibits the greatest capacity to concentrate eggs (Chaia et al., 1968; Sleight et al., 1982).

Variations in the numbers of *S. mansoni* eggs eliminated in the feces of infected individuals do not affect the determination of parasite burden or the evaluation of therapeutic interventions in populations. The estimation of intensity of infection based on a single quantitative fecal examination by the Kato/Katz method remains constant over periods of days and months. The methodology employed in the analysis of the data of a number of authors

who have evaluated the stability of eggs in the feces appears to have been fundamental in causing controversy in respect to this subject (Barreto et al., 1978; Domingues et al., 1980; Chieffi et al., 1981; Lambertucci et al., 1983, Lima e Costa et al., 1984). The reanalysis of some of these publications by determination of logarithmic function of the number of eggs and the evaluation of the results by groups employing the statistical analysis of variance, and not by comparison of individuals, show that the elimination of *S. mansoni* eggs in human feces, as measured by the Kato/Katz method is stable in all cases (Rabello, 1990).

A significant increase in the sensitivity of parasitological examination of feces can be achieved by increasing the number of samples examined (Dias et al., 1971; Torrealba et al., 1976; Long et al., 1981; Hoshino-Shimizu et al., 1986). Fig. 1 shows the increase in positivity obtained by examination of six fecal samples by the Kato/Katz method in a group on 217 individuals. In this group, which had a geometric mean of 185 eggs per gram of feces, the increase in positivity was significant up to the third sample examined (Rabello, 1990).

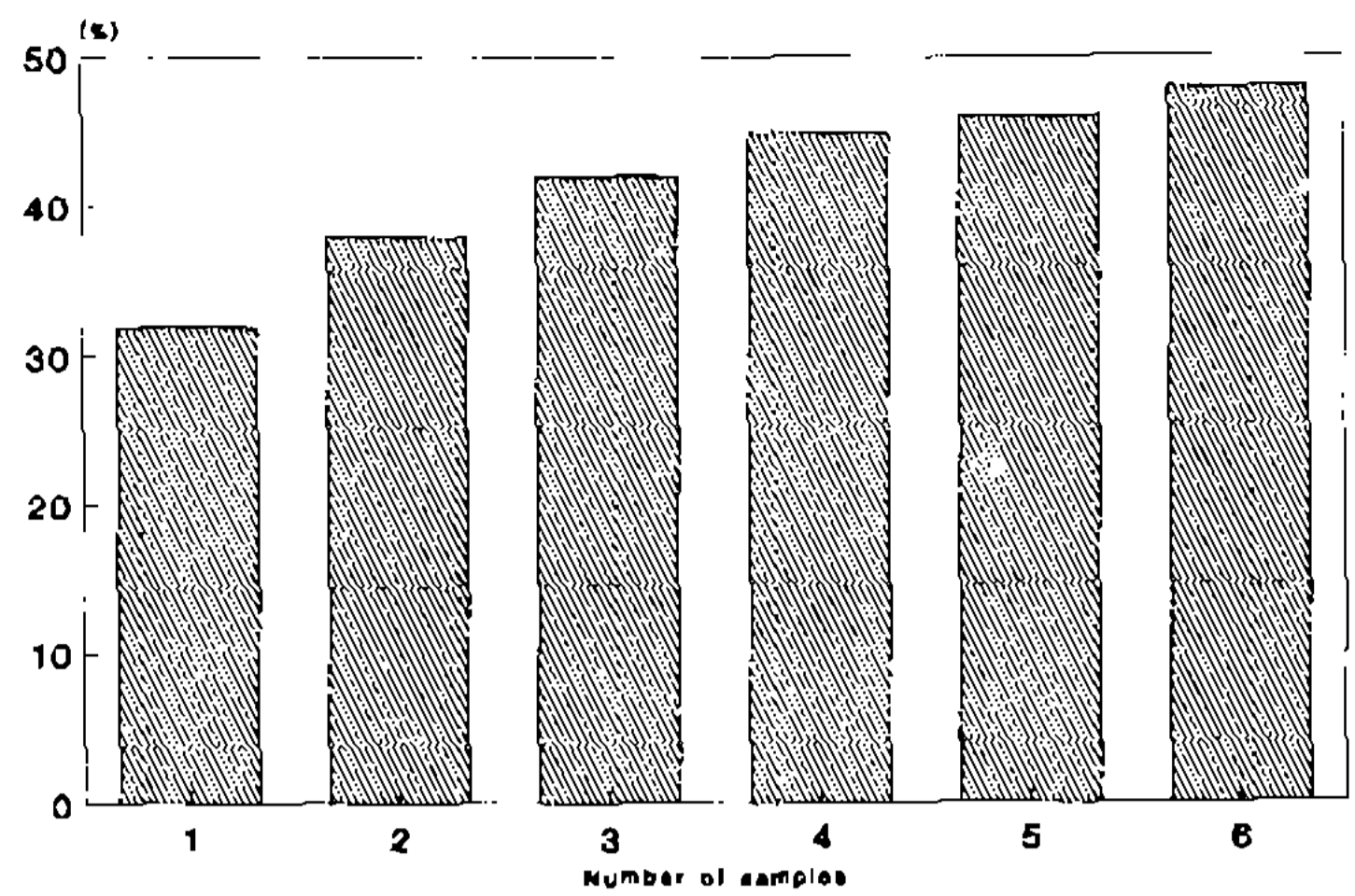


Fig. 1: cumulative frequency of *Schistosoma mansoni* diagnosis after one to six Kato/Katz stool examinations of 217 individuals.

Experiences with the examination of multiple samples permit the rational epidemiological interpretation of the diagnosis of schistosome infection based on a single sample. In an endemic area in Brazil, the increase of positivity when from one to four fecal samples taken from individuals grouped according to parasite burden were examined by the Kato/Katz method can be seen in Fig. 2. In this group of 196 randomly selected inhabitants, a single examination was statistically capable of diag-

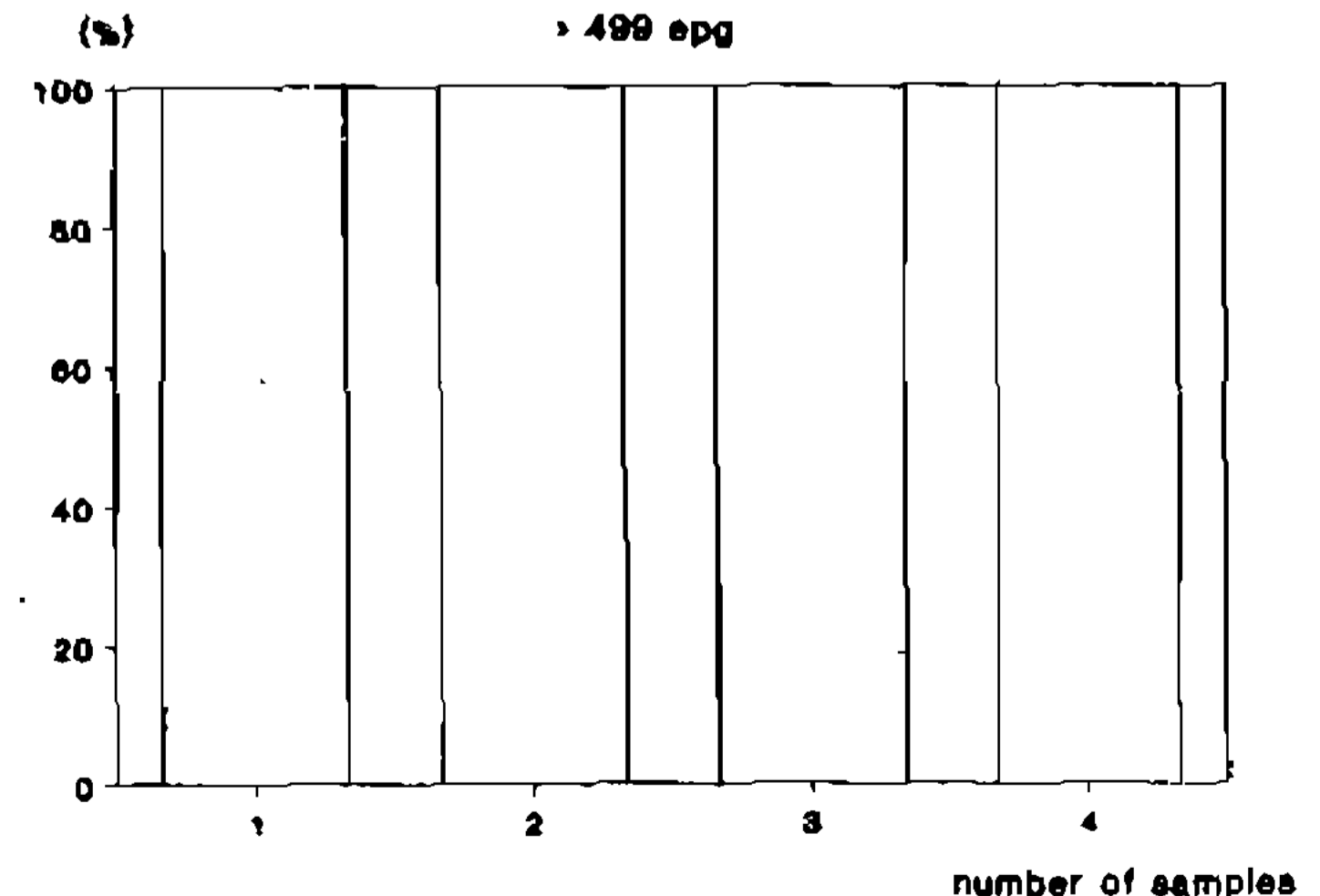
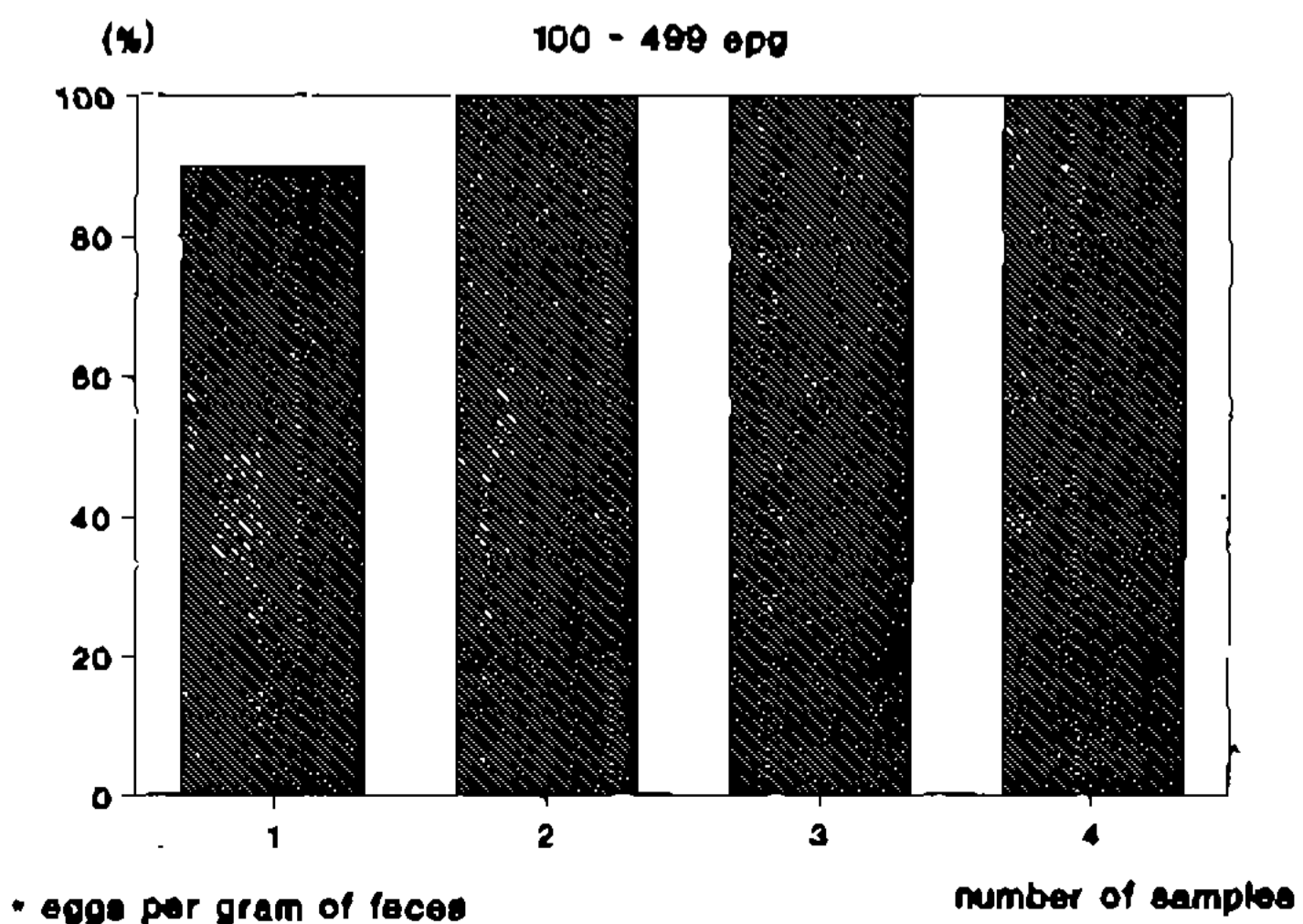
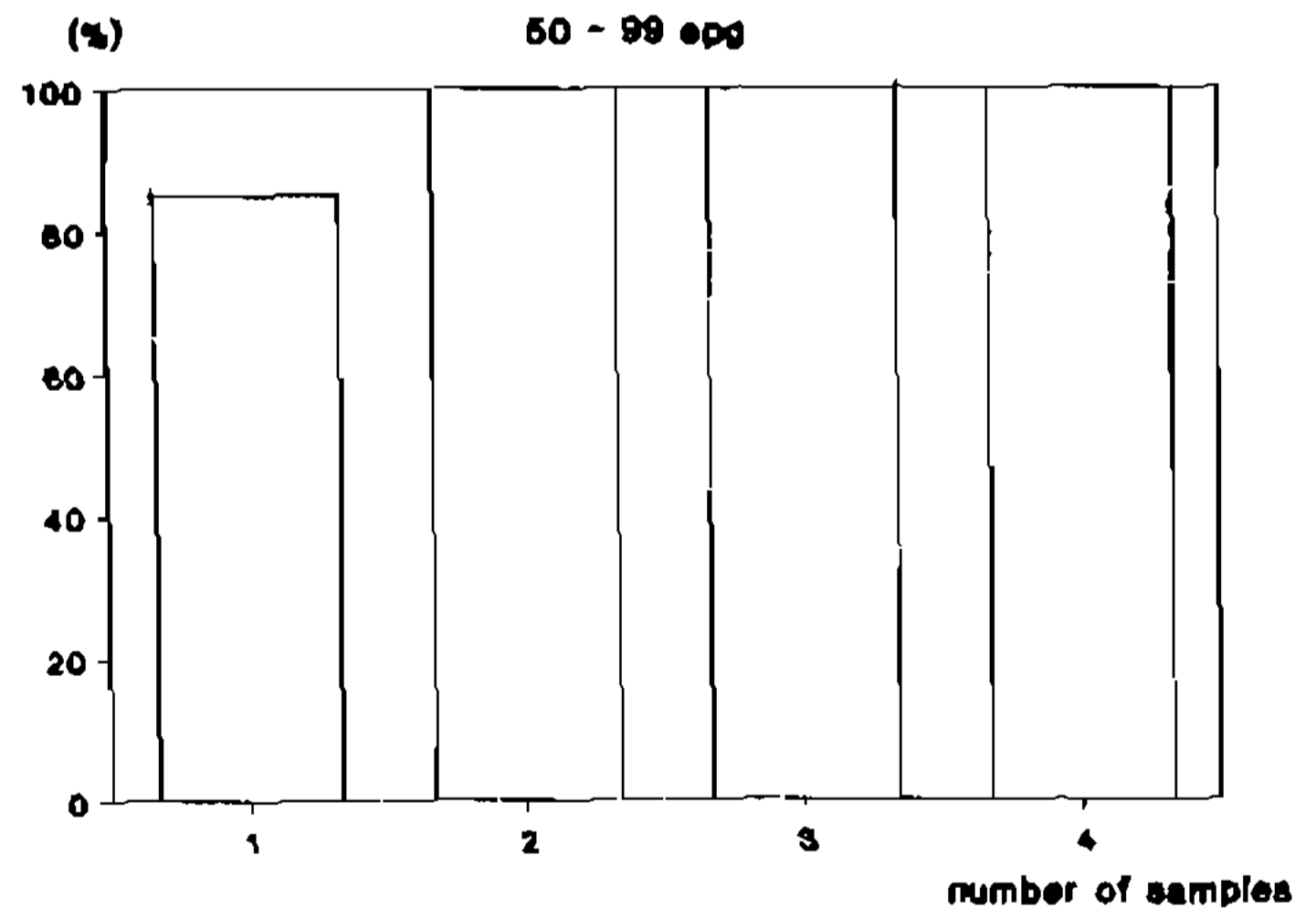
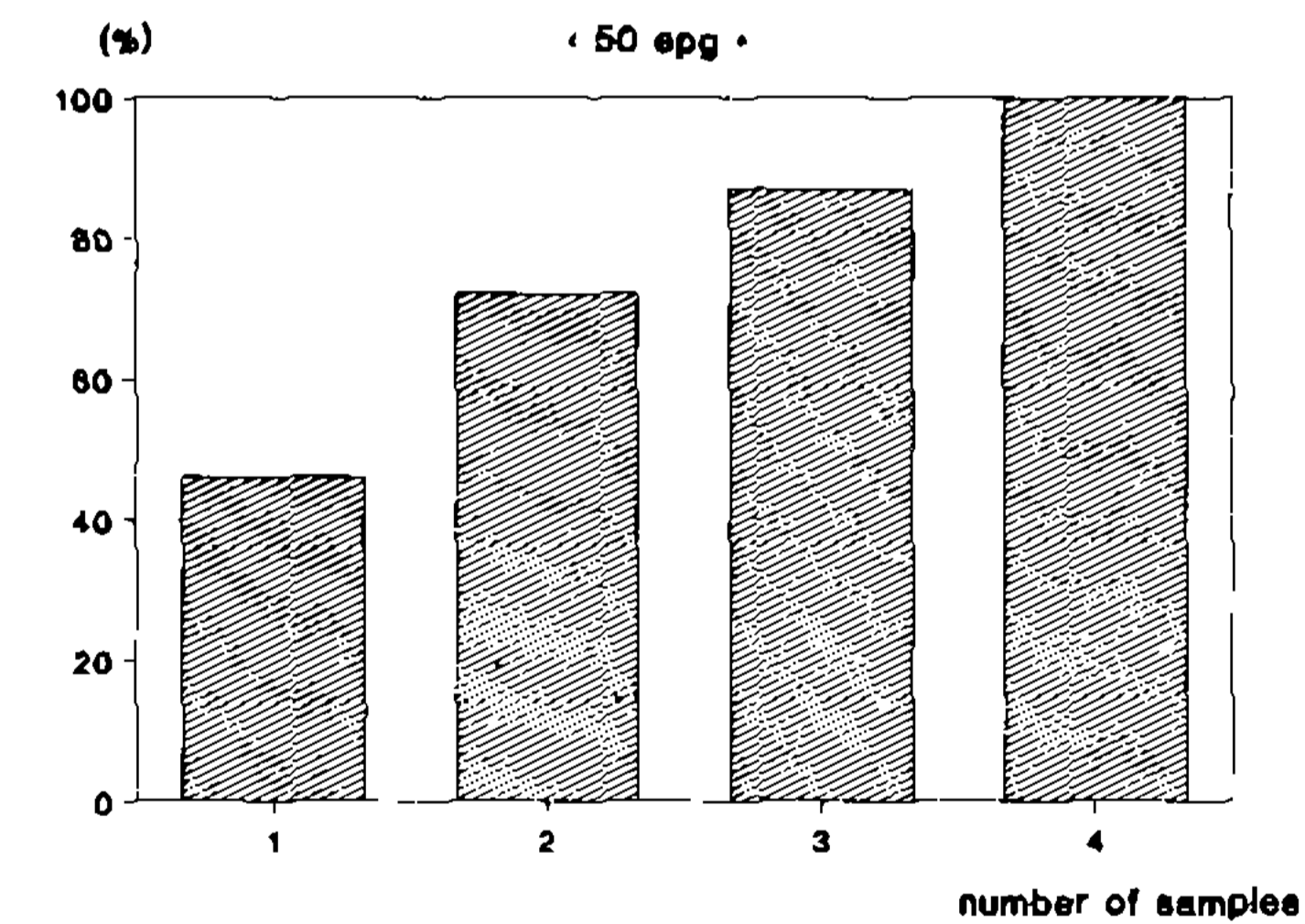


Fig. 2: sensitivity of one to four stools examinations by Kato/Katz method in 196 inhabitants of Água Branca, MG, Brasil.

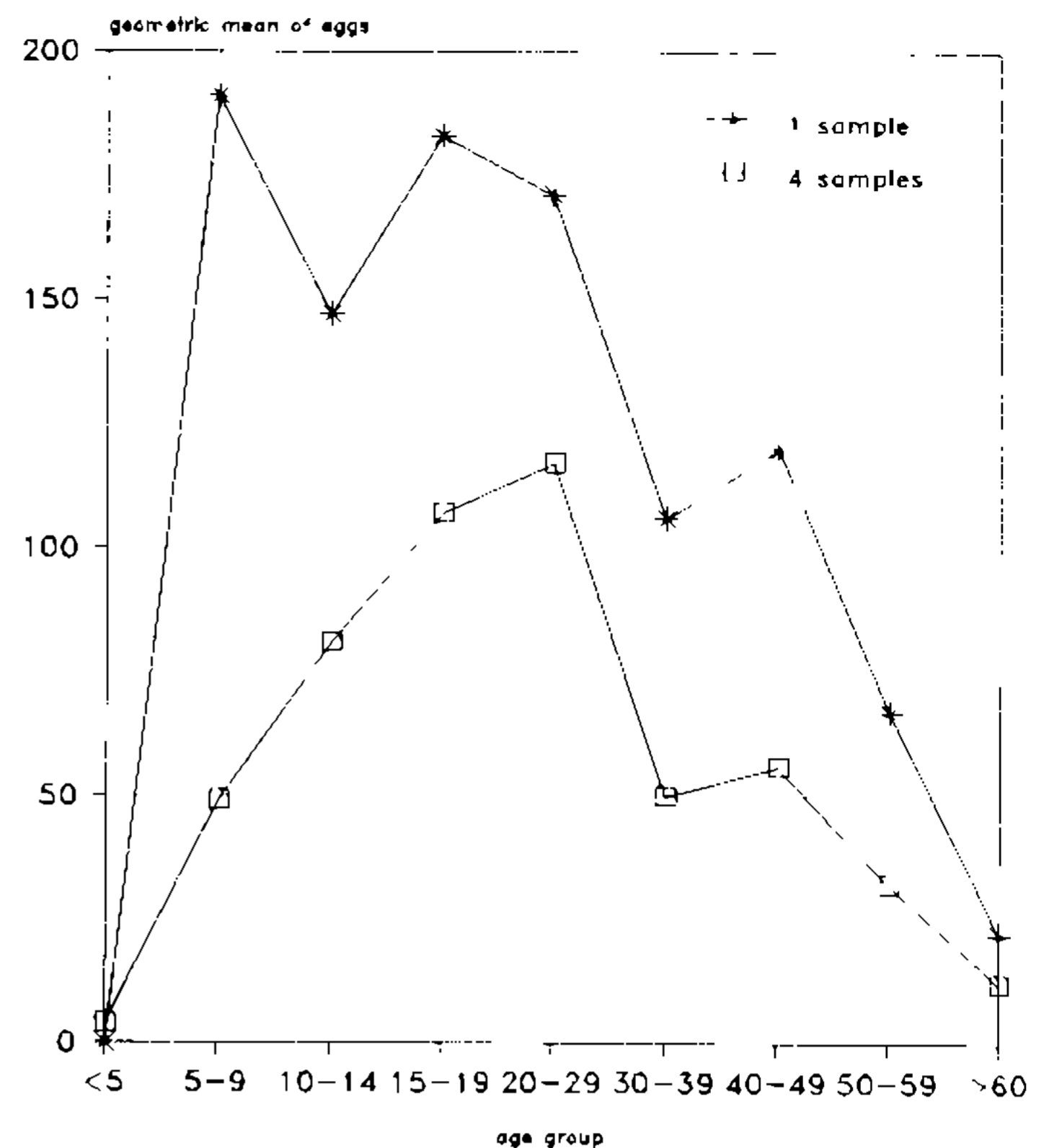
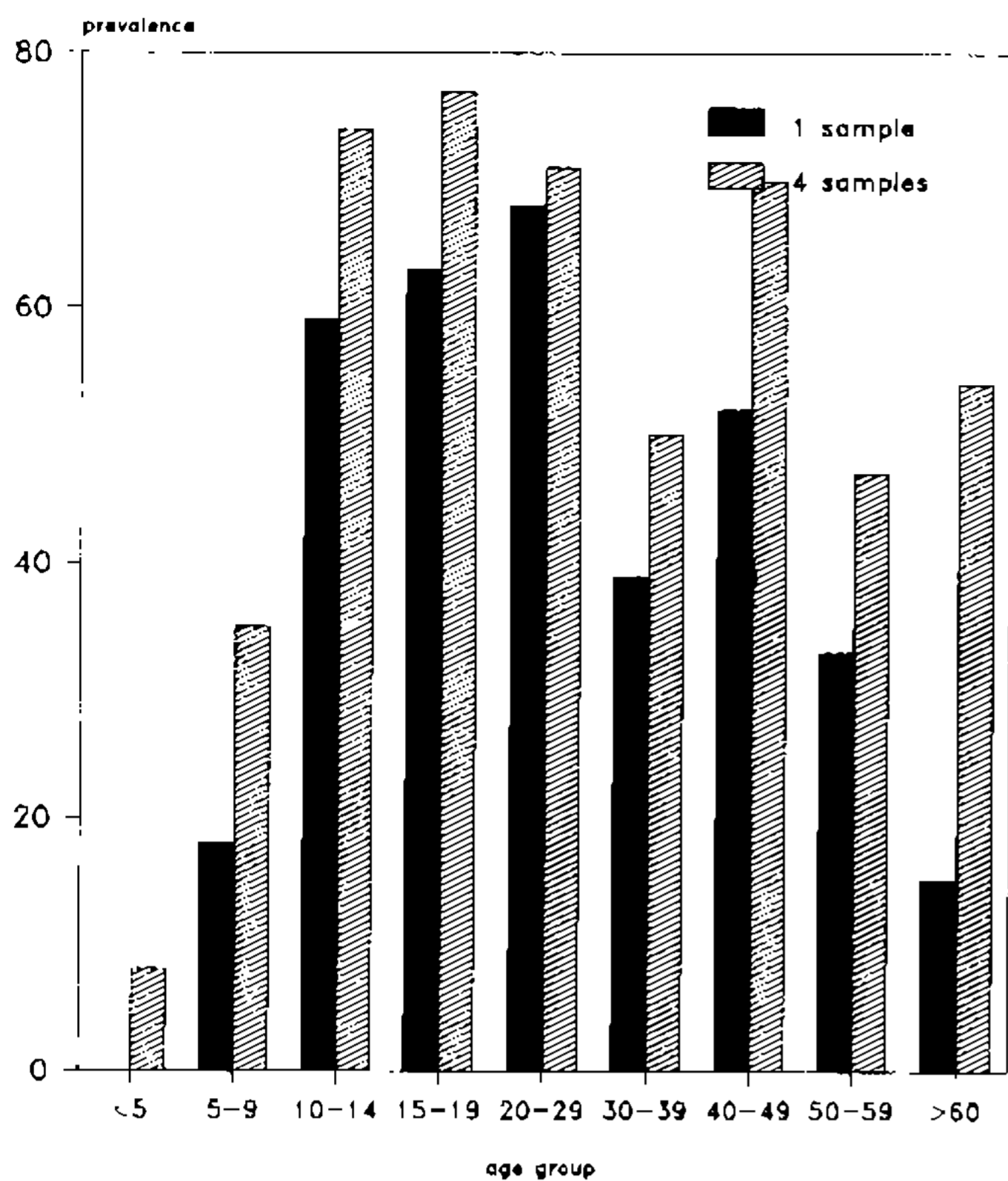


Fig. 3: prevalence and geometric mean after one and four stool examinations by Kato/Katz method in 196 inhabitants of Água Branca, MG, Brazil.

nosing the same number of infected individuals as four examinations in those having an egg output of more than 50 eggs per gram of feces.

The effect of increasing the sensitivity of the diagnosis for light infections is shown in Fig. 3. In those age groups that are normally associated with low prevalence and intensity of infection (young children and the elderly), the increase observed in these parameters when four examinations are undertaken is striking. The difference between the two curves of intensity of infection also reflects the effect of the increase in the number of lightly infected individuals diagnosed with the examination of four fecal samples.

BIOPSY OF THE RECTAL MUCOSA

As early as 1908, Pirajá da Silva referred to the finding of a large number of *S. mansoni* eggs in the rectal mucosa in a post-mortem examination. In 1943, Ottolina and Atencio introduced biopsy of the rectal mucosa for the diagnosis of schistosomiasis. Today the technique is relatively simple and can be undertaken without prior preparation, although a previous voiding of the intestine is desirable. The microscopic analysis of fragments of known weight taken from the rectal valves

allows a quantitative classification of the schistosomal elements based on developmental and morphological form from which criteria for the evaluation of anti-schistosome drugs have been established. Variations are found among the various classifications but it is agreed that the finding of live eggs is the only reliable indicator of an active infection (Vianna Martins, 1949; Prata, 1957; Cançado et al., 1965; Pellegrino & Faria, 1965; Cunha & Carvalho, 1966).

Table shows the results of a comparison between the sensitivity of rectal biopsy and from one to six fecal examinations by the Kato/Katz method for the diagnosis of schistosomiasis mansoni. A single rectal biopsy resulted in the diagnosis of more individuals than a single fecal examination by the Kato/Katz method and had a similar level of sensitivity for the diagnosis of schistosomiasis as between two and five Kato/Katz examinations. Six stool examinations by the Kato/Katz method diagnosed a statistically greater number of infected patients than the rectal biopsy. The positive correlation between the number of eggs found in the rectal mucosa and the feces (Fig. 4) confirms the reliability of counting the eggs in the feces as a measure of intensity of schistosome infection as previously demonstrated by Cheever (1968).

TABLE

Analysis of discordance between rectal biopsy and one to six stool exams by the Kato/Katz method in the schistosomiasis diagnosis, in 217 individuals

Method	Rectal biopsy				$\chi^2 a$	p
	positive	(%)	negative	(%)		
1 exam						
Positive (%)	60	(27.6)	9	(4.1)	8.02	0.005
Negative (%)		(12.4)	121	(55.8)		
2 exams						
Positive (%)	67	(30.9)	16	(7.4)	0.25	0.617
Negative (%)	20	(9.2)	114	(52.5)		
3 exams						
Positive (%)	73	(33.6)	19	(8.8)	0.49	0.486
Negative (%)	14	(6.5)	111	(51.2)		
4 exams						
Positive (%)	75	(34.6)	22	(10.1)	2.38	0.123
Negative (%)	12	(5.5)	108	(49.8)		
5 exams						
Positive (%)	77	(35.5)	22	(10.1)	3.78	0.052
Negative (%)	10	(4.6)	108	(49.8)		
6 exams						
Positive (%)	79	(36.4)	24	(11.1)	70.3	0.008
Negative (%)	8	(3.7)	106	(48.8)		

a: Mc Nemar test

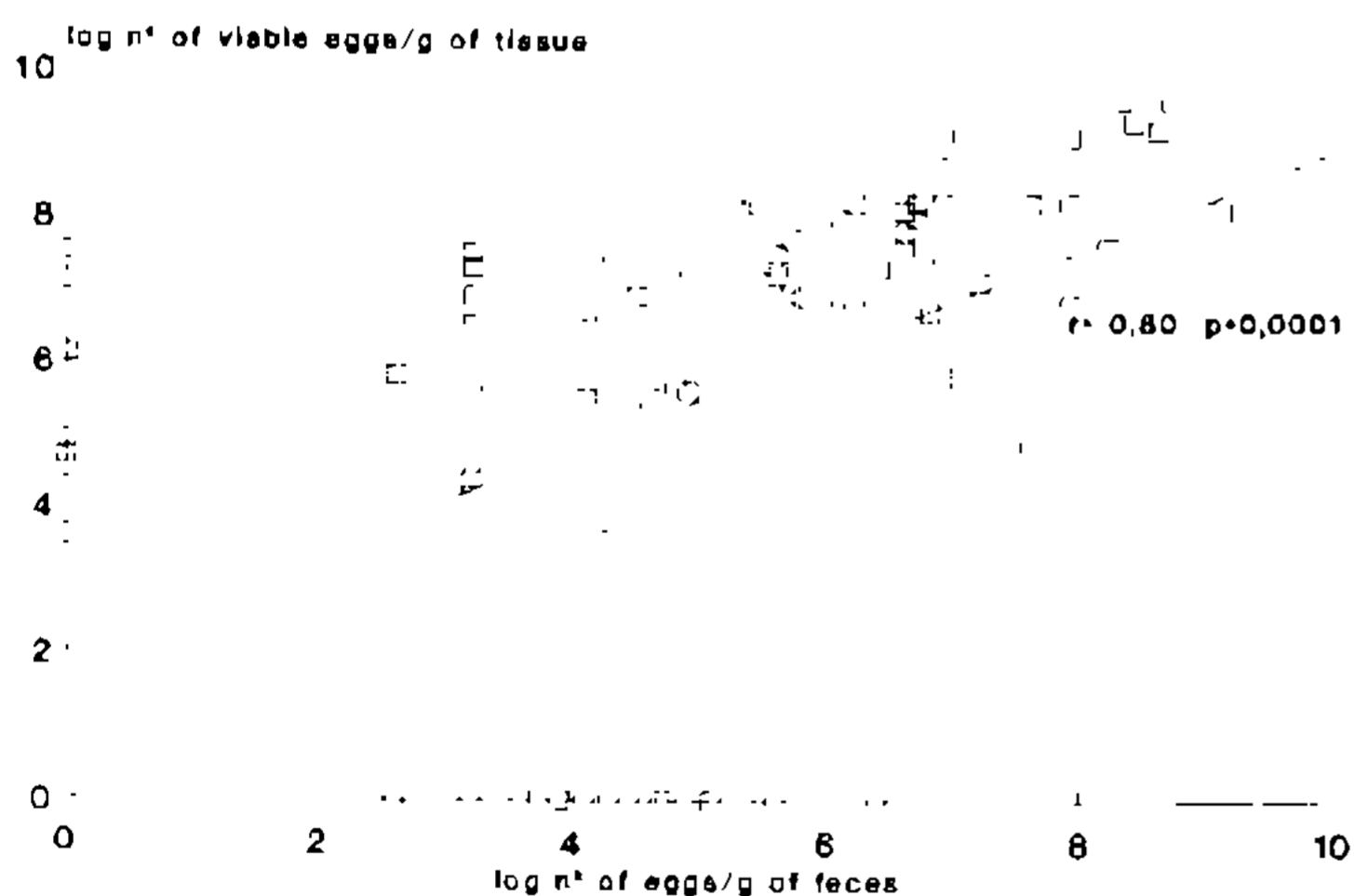


Fig. 4: correlation between number of eggs per gram of feces and number of viable eggs per gram of tissue in 217 individuals.

The use of biopsy of the rectal mucosa for the diagnosis of schistosomiasis mansoni is currently restricted to assays of new drugs when the demonstration of early modifications in the rectal oogram aids the evaluation of therapy.

In conclusion, the choice of the appropriate diagnostic method depends upon whether individuals or population are being studied. In both cases, the relatively low sensitivity of a single stool examination may be overcome by an increase in the number of fecal samples exam-

ined. For epidemiological surveys in developing countries, the Kato/Katz method is that of choice. It is quantitative, requires a short time for slide preparation and allows the slides to be easily transported for subsequent examination.

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