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EGG EXCRETION IN THE INITIAL PHASE OF EXPERIMENTAL MURINE SCHISTOSOMIASIS MANSONI: STABILITY AND ASSOCIATION WITH WORM BURDEN

Regina Lunardi ROCHA (1), Manoel Otávio da Costa ROCHA (1), Ênio Roberto Pietra PEDROSO (1), Enrico Antônio COLOSIMO (2) & Paulo Marcos Zech COELHO (3)

SUMMARY

Stability of faecal egg excretion and correlation with results related to worm burden at the initial phase of schistosomiasis mansoni were observed in two groups of mice infected with different *Schistosoma mansoni* cercarial burdens, by means of analysis of quantitative parasitological studies and schistosome counts after perfusion. Thus, it may be stated that few quantitative parasitological stool examinations could be sufficient to express the infection intensity at the initial phase, on the same grounds that it was already demonstrated at the chronic phase. Furthermore, it is confirmed that the use of the number of eggs passed in the faeces as a tool to estimate the worm burden at the initial phase of schistosome infection is adequate.

KEYWORDS: Schistosomiasis; Egg excretion; Stability; Worm burden.

INTRODUCTION

Counts of the egg's number passed in the faeces have been proposed as a useful tool for studying the epidemiology and control of parasitoses, as well as for intergroup comparison of parasitized individuals from several areas, at different intervals or under variable conditions[21]. Quantification of eggs discharged in the faeces has supported the correlation between morbidity and intensity of infection as mass treatments for schistosomiasis mansoni administered to populations from endemic areas resulted in reduction of the severe anatomo-clinical forms of the disease, associated to decrease in worm burden expressed by the number of eggs in the faeces[13].

There are two aspects of utmost interest to be considered for study of oviposition. The first aspect is based on the fact that any study dealing with estimation of eggs could be founded in just one or two quantitative stool examinations carried out at any time after infection, provided stability of egg passage in the faeces had been previously evidenced.

For this reason, stability of faecal egg excreting in *S. mansoni* infection has roused the interest of various authors for several decades, different studies on the same subject being undertaken with patients at the chronic phase of the disease[1,2,7,8,15,20,21,22]. However, stability of egg passage in the faeces at the initital phase of schistosome infection is yet to be studied.

Another aspect is related to the fact that several authors have considered the number of eggs passed in the faeces as the expression of the worm burden in schistosomiasis mansoni[6,9,10,11,12,17] although only CHEEVER (1968)[3] has verified that the passage of eggs in the faeces and the number of worms paralleled each other, in an original work using cadavers.

(1) Postgraduate Course of Tropical Medicine, School of Medicine, Federal University of Minas Gerais, Av. Prof. Alfredo Balena, 190, 30130-100, Belo Horizonte, Minas Gerais - Brazil
(2) Department of Statistics, Institute of Exact Sciences, Federal University of Minas Gerais, Brazil
(3) Department of Parasitology, Institute of Biological Sciences, Federal University of Minas Gerais - Brasil

KOURA (1970)[14] was not able to correlate the worm burden with the number of eggs detected in the faeces and tissues at the initial phase of infection. Its work, however, suffers under the consequence of the small number of animals studied, as well as for lack of statistical analysis of the results. More recently, correlation between the number of eggs voided with the faeces and the number of tissue eggs in murine infections caused by *S. mansoni* could be detected either at the initial periods or at the chronic phase of infection[4,5].

The purposes of the present investigation are to verify if there is stability in faecal egg excretion using the experimental murine model and to examine whether correlation between the number of eggs passed in the faeces and the worm burden at the initial phase of schistosome infection exists.

MATERIAL AND METHODS

Eighty female 35-day-old outbred albino mice *(Mus musculus)* were infected with *S. mansoni* and distributed into three groups, as follows: Group 1 - composed of 30 animals infected with about 15 cercariae (LE strain); Group 2 - 30 animals infected with 30 cercariae, and control group with 20 mice kept without schistosome infection.

The LE strain of *S. mansoni* used in these experiments has been maintained at the laboratories of the Schistosomiasis Research Unit, Federal University of Minas Gerais, Brazil, according to the technique by PELLEGRINO & KATZ (1968)[19]. Approximately 0.5ml suspension containing a pre-determinated number of cercariae were utilized, via subcutaneous route.

The animals were kept in individual cages, and received appropriate mouse chow and filtered water, throughout the experimental period (24 weeks).

Two quantitative parasitological stool examinations were performed per week, for 17 consecutive weeks, from day 41 post-infection onwards, in all mice from groups 1 and 2, as well as in two animals from the control group chosen at random.

Twenty-four hr-faeces were collected and, after sedimentation and homogenization, two aliquots (0.1 ml each from the total volume of 10ml, pre-determinated and standardized for all examinations) were inspected. The number of worms obtained from each slide was multiplied by 100, taking into account the arithmetical mean related to the number of worms observed on both slides.

Twenty-four weeks later, all mice from groups 1 and 2 were submitted to perfusion for counting and classification of schistosomes, as described by PELLEGRINO & SIQUEIRA (1956)[18].

STATISTICAL ANALYSIS

Stability in *S. mansoni* egg passage in the faeces received two kind of analysis depending on groups or individual infected mice were studied. In the first case, egg countings were transformed by $\sqrt{x}+\sqrt{x+1}$ (x= the number of eggs) for statistical analysis as suggested by SNEDECOR & COCHRAN[23], 1980. Comparisons between groups 1 and 2 and stability in egg excretion were examined by repeated measure analysis of variance and the least significant difference (LSD) multiple comparison of means were used when appropriated[16]. At each observation period, it was tested if the average number of excreted eggs was significantly different from the mean obtained at the anterior period.

For individual analysis, the infected animals were distributed into classes according to a low, medium or high daily egg output, corresponding to elimination of up to 300 eggs/day, 301-800 eggs/day and over 800 eggs/day, respectively.

The coefficient of variation (standard deviation/ mean x 100) was used for evaluation of stability of the faecal egg elimination in every animal submitted to observation.

Pearson's correlation coefficient and analysis of regression were utilized to study the relationship between the number of eggs passed in the faeces and the number of worms.

The level of significance considered was 5%.

RESULTS

S. mansoni eggs in the faeces could be observed from day 48 after infection onwards, a significant increase in egg elimination occurring between days 49 and 56 after infection, and reaching its peak on day 72, in both groups studied. From them on, a gradual and progressive decrease in egg elimination was observed until stabilization (Fig 1).

A significant difference occurred between the two groups in connection with the number of eggs in the faeces ($p = 0.001$). As far as worm pair is concerned, elimination of 74-124 eggs/day/worm pair could be verified in both groups studied. Stability of egg elimination occurred from 15 weeks on in group 1, and from 19 weeks onward in group 2.

Tendency toward stability appeared earlier in the group infected with a lower number of cercariae (group 1).

Individually, the average number of eggs passed in the faeces from 155 to 427 eggs/day for group 1 and from 172 to 1057 eggs/day for group 2. Taking into account all examinations performed, none of the mice remained stable in relation to egg elimination. However, in the majority of cases, changes in classes occurred to adjacent classes.

Variation coefficients ranged from 45.9% to 81.5%. Parasitological examinations with negative results occurred mostly in group 1.

Correlation coefficient between the number of eggs passed in the faeces and the number of worm pairs

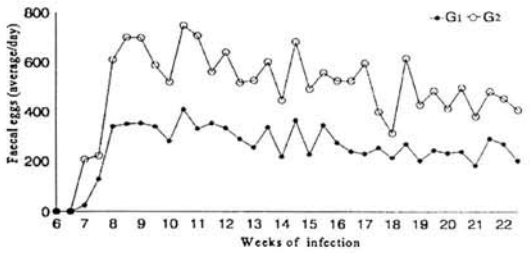

Fig. 1 - *S. mansoni* eggs passed in the faeces from two groups of mice infected with 15 (group 1) and 30 (group 2) cercariae.

TABLE

Percentual of negative parasitological stool examinations in two groups of mice infected with 15 and 30 *S. mansoni* cercariae

Group	Parasitological stool examination		
	n	Negative	%
1	544	44	8.1
2	870	60	6.9

recovered at perfusion was obtained for both groups studied (Fig. 2 and 3). This association was found to be more accentuated in group 1 ($r = 0.9430$; $p = 0.001$) than in group 2 ($r = 0.7130$; $p = 0.001$).

The number of worm pairs could explain about 89% variability in the number of eggs eliminated in the faeces from mice pertaining to group 1 and about 51% variability in the animals from group 2.

DISCUSSION

Stability of egg-laying at the chronic phase of schistosome infection has been observed by several authors. SCOTT (1938)[21] was able to follow up three Egyptian peasant boys by means of quantitative stool examinations (Stoll's method) performed for 30 consecutive days. He found a coefficient of variation around

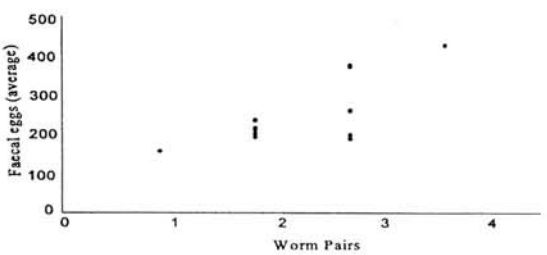

Fig. 2 - Correlation between the number of *S. mansoni* eggs passed in the faeces and the number of worm pairs recovered at perfusion (group 1).

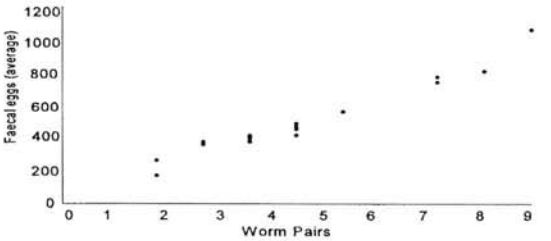

Fig. 3 - Correlation between the number of *S. mansoni* eggs passed in the faeces and the number of worm pairs recovered at perfusion (group 2).

40%, and concluded that variability in the elimination of *S. mansoni* eggs in faeces was not significant. COOK et al. (1974)[7] carried out a study of morbidity in schistosomiasis mansoni in 142 children from an endemic area. Based on quantitative stool examinations, these authors verified that all the patients kept the same intensity of infection for 2-4 years after the initial evaluation. This relatively constant state of the infection suggested that many areas could be studied through cross-sections. ARAP SIONGOK et al. (1976)[1] analysed stability in egg excretion in 416 individuals bearing schistosome infection through two quantitative stool examinations performed at 3-week-intervals. It was verified that 68% of the individuals remained within the same picture of the first examination. BARRETO et al. (1978)[2] studied stability in *S. mansoni* egg elimination in 23 individuals living in a rural endemic area in northeastern Brazil, where the prevalence was 87% by means of quantitative stool examinations (Kato's method) performed for 3-4 consecutive days/month, for a 3-month-period. Data obtained showed a large variation in a short lenght of time (3 consecutive days in each 3-month-period), the coefficient being maintained around 70%. However, when countings were analysed throughout the experimental period (3 consecutive months), the relatively stable egg elimination was evidenced by using either the gross data or the transformed ones. This stability was also observed in a rural community (with moderate prevalence) in Kenya, by SMITH et al. (1979)[22]. These authors were able to repeat stool examination in 24% of the original population, two months after the first examination. It was verified that 70% of the individuals were kept into the same category with the same characteristics.

COSTA et al. (1984)[8] carried out three quantitative stool examinations (Kato-Katz method) for a 11-week-period. They verified that Pearson's coefficient of correlation was found to be high and statistically significant, when the results of the first examination were compared with the mean obtained with the three examinations, thus showing that *S. mansoni* egg countings (expressed by geometric mean) presented high stability level. RABELLO (1990)[20] observed stability in daily *S. mansoni* egg elimi-

nation related to a group of 103 patients, by means of 6 examinations for a 6-10-day-period (Kato-Katz method). Nevertheless, LAMBERTUCCI et al. (1983)[15] did not succeed in observing the same event. These authors followed up 38 school children bearing schistosomiasis mansoni, for 10 months. They performed two stool examinations (in two consecutive days) per month, utilizing the Kato-Katz method. Their patients showed a coefficient of variation of 103%, only 28.9% remaining stable throughout the observation period. It is possible that discrepancy of the results obtained by the last authors could stem from utilization of the so called "classes of egg laying" and an inadequate statistical model.

In this experiment, mice were parted according to egg laying class, and submitted to examination individually. None of them remained stable throughout the experimental period. Variation coefficient ranged between 45.9% and 81.5% with an average of 68.7% for all the group. Usually, changes occurred to adjacent classes.

Tendency to stability was evidenced from 15 weeks after infection onward for the animals infected with 15 cercariae, and from 19 weeks on for those infected with 30 cercariae, when analysis was carried out per group and with transformed data.

Week after week, follow-up of the data related to elimination of eggs showed a marked relative stability in group 1 throughout the initial phase, and not only from 15 weeks after infection onwards. In this group of animals, significant alteration in the number of eggs passed in the faeces occurred at 7 weeks, when a marked increase was observed, and between 14 and 15 weeks, when a significant decrease was detected. Therefore, a first stable phase could be observed between 8 to 13 weeks, with a high elimination rate, as well as a second stable phase, with a low egg elimination in the faeces, from 15 weeks after infection onwards.

Conversely, these two stability phases could not clearly be observed in group 2 (more heavily infected).

As expected, the more intense the infection, the more scarce the occurrence of negative parasitological examinations. However, contrary to expectation, stability in egg elimination was more evident and occurred earlier in the group presenting a higher rate of negative parasitological examinations.

The results obtained showed, for the first time, that the number of eggs excreted in the faeces bears correlation with worm burden at the initial phase of schistosomiasis. This finding constitutes the experimental basis for utilization of the number of eggs passed in the faeces as estimation of the worm burden, at the earlier stages of schistosome infection too, as already done by several authors[6,9,10,11,12,17], by analogy with data obtained at the chronic phase of infection.

Furthermore, observation connected with tendency to stability of faecal egg excretion at the initial phase of murine schistosomiasis suggests that it is possible to estimate earlier the infection intensity, by means of examinations carried out with few faecal samples.

RESUMO

Esquistossomose mansoni experimental murina: estabilidade da eliminação dos ovos nas fezes e sua associação com a carga parasitária na fase inicial da infecção

Através da análise de exames parasitológicos quantitativos seriados e da contagem de esquistossomos após perfusão em dois grupos de camundongos infectados com diferentes cargas de cercárias de *Schistosoma mansoni*, verificou-se a existência da estabilidade da eliminação de ovos e sua correlação com a carga parasitária na fase inicial da esquistossomose mansoni.

Deste modo, pode-se afirmar que poucos exames parasitológicos de fezes quantitativos podem ser suficientes para traduzir a intensidade da infecção também na fase inicial , à semelhança do já demonstrado para a crônica.

Além disto, comprova-se a adequação do uso do número de ovos eliminados nas fezes como expressão da carga parasitária na fase inicial da infecção esquistossomótica.

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