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A CRITICAL EVALUATION OF THE EXPRESSION OF PARASITEMIA IN EXPERIMENTAL CHAGAS' DISEASE

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SUMMARY

This paper aims to study the best way to express the parasitemia of *Trypanosoma cruzi's* experimentally infected animals. Individual scores may have a great variability, not emphasized by the majority of the authors. A group of 50 rats infected with $1x10^6$ trypomastigotes of *T. cruzi* Y strain was used and the parasitemia was estimated by BRENER's method. The results showed that the median can avoid false results due to very high or low parasitemias but it does not have the mathematic properties necessary for analysis of variance. The comparison of the means of the original and transformed data, with their respective coefficients of variability (CV), showed that the logarithmic mean (Mlog) have the minor value of CV. Therefore, the Mlog is the best way to express the parasitemia when the data show great variability. The number of the animal for group did not affect the variability of data when the Mlog and CV were used.

KEY WORDS: *Trypanosoma cruzi;* Parasitemia; Critical evaluation.

INTRODUCTION

During his early observations on chagasic patients and on animals naturally or experimentally infected with *T. cruzi*, Carlos Chagas [3] already pointed out the variability of the number of trypomastigotes that occurred during infection development.

Nowadays, blood parasitemia is an important parameter for the study of Chagas' disease, since it allows us to differentiate between the acute and chronic phases of infection. It is also necessary to establish anatomo-pathological correlations and monitor the patients progress towards cure. Blood parasitemia is used as a good biological marker for the characterization and establishment of different strains of *T. cruzi*.

The methods usually used to estimate the level of parasitemia vary considerably. They range from the researcher subjective evaluations [8, 14] to counting of the trypomastigote numbers either per time of examination [5] or per known volume of infected blood [2, 9].

There are, however, some questions which often come to the investigators'mind when estimating the extent of parasitemia, such as: 1. What is the best way to express numerically the parasitemias? 2. How should one express the great variability of the results obtained within a group of observed animals? 3. Does the difference in the number of sampled animals influence the results in experiments of this nature?

Usually, the authors express the parasitemia by the mean number of parasites per animal within a group;

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occasionally they make use of the median value [6, 10]. However, only some of the authors draw attention to the variability observed for a particular data set, with the inclusion of the standard deviation or percentile values in the results presented [4, 13].

So far, no comparative studies have been done on the measures of central location that may best represent the parasitemia and on their degree of variability. The ideal number of animals to be sampled for experiments of this nature is not known.

The present work was undertaken in order to: 1. Compare the expression of the parasitemia using the arithmetic mean of the original counts or of the transformed data, and the median; 2. Try to establish the ideal number of animals to be used in such experiments.

MATERIAL AND METHODS

Animals: A set of 35 ± 1 days old albino Wistar male rats (n=50) weighting 100-110g was used in the experiment. The animals were obtained from the Main Animal House of the UNESP, Botucatu-SP.

Infection: Rats were inoculated by intraperitoneal route, with 1 x 10^6 trypomastigotes of *T. cruzi* Y strain[12]

Parasitemia: Daily individual parasitemias were determined using blood collected from the animal's caudal vein, as previously described by BRENER[2].

Statistical analysis: The data related to the 50 rats' parasitemia was expressed using the arithmetic mean of the original values obtained (M), or by their square root (Mq) and logarithmic (Mlog) transformations. The median of the original counts (Md) or their respective square root (Mdq) or logarithmic (Mdlog) tansformations, were also tested as measures of central location. The variability of the data set was given by the standard deviation(s) and coefficient of variability (CV). Confidence limits at 95% of CV were calculated for the means (M, Mq, Mlog), and the degree of variability of the median was expressed by the percentiles (P25 and P75).

Influence of the sample size on the results obtained: In order to do this, the parasitemia was estimate in the original set of 50 rats, divided into 4 groups of either 3, 5, 8 or 10 animals, each group being run in triplicates. The distribution of rats per group was done at random using the program for the generation of randomic numbers of the calculator HP-11C. The mean for each group was calculated, after logarithmic transformation of the original values, and both the standard deviation and the coefficient of variability were estimated. The data's statistical analysis was done comparing the coefficient of variability between groups according to the Kruskal-Wallis test [7] for independent samples, for p=0.05.

RESULTS

Blood parasitemias of the 50 rats infected with *T. cruzi* were expressed using at first the distribution of the individual original values obtained, the M and standard deviation (Fig. 1). Comparison between the M and Md with the percentils P25 and P75 were expressed by original values in the Figure 2. The Figure 3 shows the comparison between Mq versus Mdq and Mlog versus Mdlog. The coefficients of variability fo each case (CV, CVq, CVlog) ae represented in Figure 4.

The statistical analysis of the data related to the influence of sample size on the variability, given by the CV, did not detect significant differences for p=0.05 among the different groups of rats analysed.

DISCUSSION

We observed standard deviation and CV values of up to 494% when the M of the original counts registered were used to express parasitemia, despite having analysed a large number of experimental animals (n=50). This indicates that the data obtained shows a high degree of variability (Fig. 1). In order to consider the M as a good measure of central location, neither the standard deviation nor the CV can be high. CV values of up to 25% are considered standard or habitual in

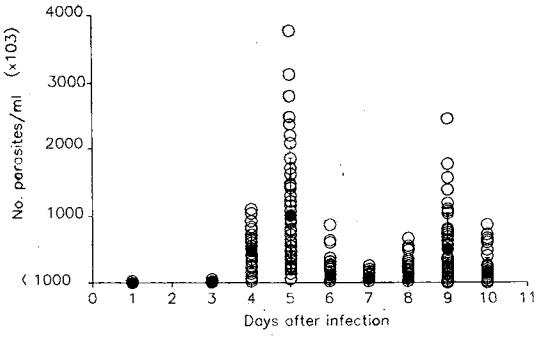

Fig. 1 - Distribution of the original (○) and the mean and standard deviation (●) values of parasitemia in 50 rats with *T. cruzi*.

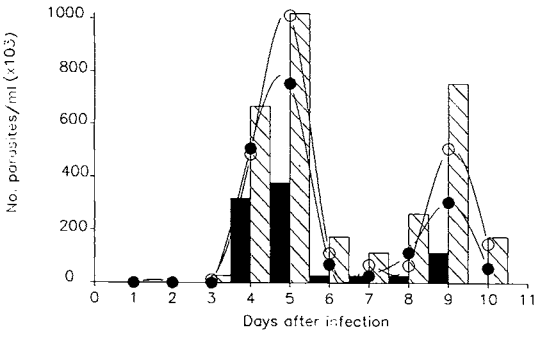

Fig. 2 - Comparison of values of parasitemia in rats infected with *T. cruzi*: mean (O——O), median (●——●) and percentils 25 (▪) and 75 (▨).

biological experiments [1]. Hence, a data set with a high CV, as is in the present experiment, does not confer credibility to the conventional M to be used as a tool in the statistical analysis.

On the other hand, when comparing the M and the Md of the original values obtained (Fig. 2) we found that those for the former are greater. It demonstrates once more the influence of high individual counts on the arithmetic mean of parasitemia. After either square root or logarithmic transformation of the values, there is a decrease in the differences between the M and Md (Fig. 3), indicating that both transformations reduce the influence of aberrant values of the sample. When comparing the CV calculated using the original numbers with those of the transformated ones (Fig. 4), we observed that the smallest CV was obtained using the logarithmic transformation of data (CVlog). The results indicate that, concerning parasitemia, the Mlog of the values can be used as a safe and useful parameter to express the central location of data set. This fact suggests that when the data set has a skew distribution, as is the case for parasitemia, it is not appropriate to refer to the M of the original numerical values. It is thus necessary to make use of some transformation of the numbers initially recorded.

Attempts to use the Md as a measure of central location showed that it gives a reasonable graphical representation of the data, but it does not have the mathematical properties necessary for an analysis of variance. Furthermore, the expression of the parasitemia as absolute numbers of both, the Md and the percentiles, does not allow a comparison between different experiments, since most authors make use of M values. On the other hand, the use of the Mlog has two advantages: a) it reduces significantly the degree of variability allowing the use of all the mathematical properties inherent to the mean; b) it permits an analysis of variance for the comparison between groups. Preliminary studies [13] suggested that the Md value could be adequate parameter for the expression of parasitemia. However, a critical analysis of the results obtained in the present work suggests that the Mlog, together with the standard deviation and CVlog, is more adequate for expression of parasitemia data. In this way, it is possible to make a statistical comparison of the differences and similarities between parasitemias. From a practical point of view, whenever a researcher uses Brener's method [2] for trypomastigotes quantification, it will be necessary to make a new table were each computed result would be added by a factor of 10, followed by a subsequent logarithmic transformation.

This work stablished that the Mlog can be safely used to express the parasitemia of rats infected with *T. cruzi*. Considering that in experiments of this nature it is not usual to utilize such a large number of animals, as the one used in the present work, the CVlog were used to analyse the influence of the number of animals per

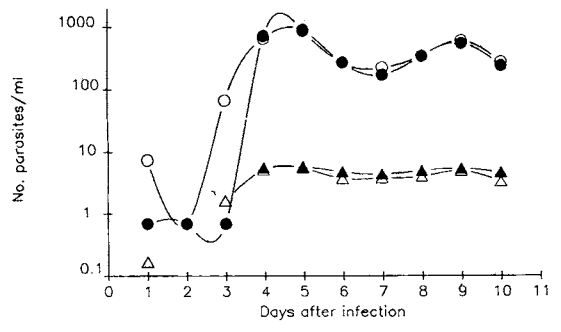

Fig. 3 - Comparison of values of parasitemia in *T. cruzi* infection expressed as: Mq (O——O), Mdq (●——●), Mlog (Δ——Δ) and Mdlog (▲——▲).

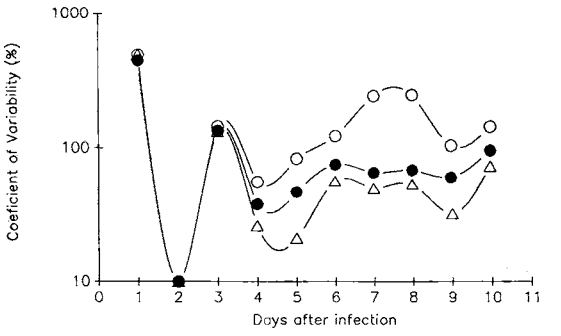

FIG. 4 - Comparison of CV (%) values of parasitemia in rats infected with *T. cruzi*, expressed in the original values (O——O), with square root (●——●) and logarithmic (Δ——Δ) transformations.

group on this measure of variability. The statistical analysis of the CVlog did not indicate any significant differences among the groups made up of either 3, 5, 8 or 10 rats. These results suggest that a small number of animals can be used in experiments, if the parasitemia values are expressed in Mlog, despite of the several known factors influencing the counts of parasitemia in *T. cruzi* infection [11, 15].

RESUMO

Estudo crítico sobre a expressão da parasitemia na doença de Chagas experimental

O objetivo deste trabalho foi o de estudar a melhor maneira de expressar a parasitemia de animais experimentalmente infectados com *T. cruzi*, visto que os animais podem apresentar grande variabilidade nos dados de parasitemia e que não é expressa nos resultados da maioria dos autores. Nós usamos um grupo de 50 ratos que foram infectados com $1x10^6$ tripomastigotas da cepa Y de *T. cruzi* sendo a contagem dos parasitas feita pelo método de BRENER. Os resultados mostraram que a mediana pode impedir falsos resultados devido a valores muito altos ou muito baixos na parasitemia, porém não contém propriedades matemáticas que permita usá-la em inferência estatística. As médias, com dados originais e transformados, foram comparadas entre si através dos respectivos coeficientes de variação (CV). Os resultados mostraram que a média logarítmica (Mlog) tem os menores valores de CV, sendo o melhor parâmetro para expressar a parasitemia quando os dados apresentam grande variabilidade. Estudamos também a influência do número de animais sôbre a variabilidade de dados usando a Mlog e o CVlog de grupos formados por 3, 5, 8 e 10 ratos; a análise estatística feita pelo teste de KRUSKAL-WALLIS não mostrou diferenças significantes entre os grupos.

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