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Prevalence of *Tetratrichomonas didelphidis* from the Cecum of the Opossum *Didelphis albiventris* in the Botanical Garden, Porto Alegre, Rio Grande do Sul, Brazil

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ABSRACT

Tetratrichomonas didelphidis (Hegner and Ratcliffe 1927) Andersen and Reilly 1965 is a flagellate protozoan from the intestine, cecum and colon of *Didelphis marsupialis* Linnaeus, 1758. The prevalence of *T. didelphidis* in opossums *D. albiventris* was studied in the Botanical Garden, Porto Alegre City in the southernmost Brazilian State, Rio Grande do Sul, Brazil. *T. didelphidi* was found in seven of eight cultures of swabbed rectums, representing a prevalence of 87.5% in *D. albiventris.* In the present investigation it was observed that the *T. didelphidis* found in the intestine content of *D. albiventris* had the same morphological characteristics as those previously described by other authors in the *D. marsupialis*, being the same protozoan species in both host species.

INTRODUCTION

Tetratrichomonas didelphidis is a parasitic protozoan from the intestine, cecum and colon of the opossum *Didelphis marsupialis*. The protozoan is a flagellate belonging to the family Trichomonadidae, subfamily Trichomonadinae (Honigberg 1963). The taxonomy of trichomonads has had a complex history. This species was rust described by Hegner and Ratcliffe

(1927) with the name *Trichomonas didelphidis*. Subsequently, Andersen and Reilly (1965) described again the species on the correct genus Tetratrichomonas.

Up to the present there is only one paper on the occurrence and anatomy of trichomonads from opossum *D. marsupialis*. There is no paper about the prevalence in *D. albiventris* and this is the first report on the occurrence of *T. didelphidis* from the cecum and colon in opossums (*D. albiventris*). Nothing is known about the transmission, epidemiology, pathogenicity, biochemistry and immunologic features, neither about the culture requirement of the parasites. The aim of this study was to determine the prevalence of *T. didelphidis* in opossums *D. albiventris* in the Botanical Garden, Porto Alegre City in the southernmost Brazilian State, Rio Grande do Sul, Brazi1.

MATERIAL AND METHODS

In the present study was used the Diamond's modified trypticase-yeast extract-starch (TYS) medium, pH 7.5, without maltose and with starch solution (5 mg/m1) (Tasca et al. 1999). The opossums were captured and, immediately after examination set free with a little incision on the ear for demarcation to avoid repetition of animals. The rectum of the opossums was swabbed and the swabbs and fecal material was inoculated into TYS medium, pH 7.5, for incubation. Samples were cultured in vitro at a temperature of 28 ^{o}C (±0.5) in TYS medium supplemented with 10 % (v/v) heat inactivated bovine serum, penicillin (1000 U/m1) and streptomycin sulfate (1 mg/m1). Material for the stained smears was obtained from a 72 h old culture in TYS medium and stained by the Giemsa method (De Carli et a1. 1979). The morphologic study was made by light microscopy and all photomicrographs were made with an Olympus AX 70 photomicroscope connected to a video camera and to a computer with the program Image-Pro Plus 4. 1.

RESULTS

The prevalence of *T. didelphidis* was determined in the summer, January and February. *T. didelphidis* was found in seven of eight cultures of rectums swabbed, representing a prevalence of 87.5% in opossums *D. albiventris*. The number of opossums investigated in the park was 8 (n

= 8), based on the binomial probabilities table (Kachigan 1986) and, on the occurrence data of other authors (Andersen and Reilly 1965). Direct microscopic examination of wet smears from the intestinal content and feces revealed vast numbers of actively motile flagellated protozoa. The organisms were classified as trichomonads because of their elongate ellipsoid shape and the presence of an undulating membrane associated with four free anterior flagella which could be accurately counted only when the trichomonads had slowed down or stopped moving. The body is very plastic but not particularly amoeboid. The trophozoites had a long posterior free flagellum and a parabasal body disc-Shaped with well defined constant central granule. Most of these morphological features could be recognized in air-dried smears of intestine content, fixed in methanol and stained with Giemsa. Examination of the cultures after incubation at 28 ^{o}C from 48 to 72 h revealed a heavy growth of flagellates. The cultures were routinely maintained in TYS medium at 28 ^{o}C and transferred three times per week (at 48 or 72 h). Even so, in this investigation it was observed that trophozoites of *T. didelphidis* had the same morphological characteristics as previously described by other authors.

DISCUSSION

The trichomonads belong to the subkingdom Protozoa, phylum Sarcomastigophora, subphilum Mastigophora, class Zoomastigophorea, superorder Parabasalidea and order Trichomonadida (Levine et al. 1980). They are classified in the family Trichomonadidae and subfamilies Trichomonadinae, Tritrichomonadinae and Pentatrichomonadinae. In the subfamily Trichomonadinae there is a typical pelta. This subfamily contains four genera. In the genus *Trichomonas* the posterior flagellum is not free, whereas it is in the other three; *Trichomonas* has four anterior flagella. The other three genera can be differentiated by the number of their anterior flagella and by the shape of their parabasal bodies. In the genus *Trichomitus* there are three anterior flagella and the parabasal body is usually V-shaped (occasionally rod-shaped). In the genus *Tetratrichomonas* there are four anterior flagella in mature individuals and the parabasal body is usually disc-shaped. In the genus *Tetratrichomonas* there are four anterior flagella, of which four are grouped together at the base and one is independent, and the parabasal body appears to be composed of small granule(s) (Honigberg 1963).

The genus *Tetratrichomonas* is different from *Trichomonas* not only with respect to minor morphological characteristics but also in the structure of the well-developed undulating membrane, the outer margin of which continues into a free posterior flagellum. It is the last attribute which facilitates most the distinction between *Trichomitus* and T*etratrichomonas* on one side and *Trichomonas* on the other. Although it is nearly impossible to be certain which of the genera, *Tetratrichomonas* or *Trichomonas*, or if both of them, came directly from a *Trichomitus*-type flagellate, it seems that in most features, morphological as well as physiological (for example, the primitive site in the hosts), *Tetratrichomonas* is closer to the main line of evolution (Honigberg 1963). The phylogenetic analysis of *T. didelphidis* is in the present, aim of study of our group.

The measurement of our material was compared to the results of others (Andersen and Reilly 1965) with the intent to identify possible variation. In the present investigation it was observed that *T. didelphidis* found in the intestine content of opossums *D. albiventris* had the same morphological characteristics as those previously described by Andersen and Reilly (1965) in *D. marsupialis*, being the same protozoan species in both different host species.

As shown by our survey, this trichomonad is a frequent inhabitant in the intestine of opossums. Andersen and Reilly (1965) observed that the optimum location of *T. didelphidi*s is the cecum. This distribution within the host is probably related, in part, to the pH of that area.

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REFERENCES

Andersen, F. L. & Reilly, R. 1965. The anatomy of *Tetratrichomonas didelphidis* (Hegner and Ratcliffe, 1927) *comb. nov.* from the opossum. *J. Parasitol.* 14:27-35.

De Carli, G. A., Pansera, M, C. G. & Guerrero, J 1979. *Trichomonas gallinae* (Rivolta, 1878) Stabler, 1938, no trato digestivo superior de pombos domesticos, Columba livid, no ho Grande do Su1 - Primeiro Registro. *Acta Biol. Leopoldensia* 1:85-95.

Hegner, R. W. & Ratcliffe, H 1927. Trichomonads from the vagina of the monkey, from the

mouth of the cat and the man, and from the intestine of the monkey, opossum and prairie-dog. *J. Parasitol.* 14:27-35.

Honigberg, B. M. 1963. Evolutionary and systematic relationships in the flagellate order Trichomonadida Kirby. *J. Protozool.* 10:20-63.

Kachigan, S. K. 1986. Statistical analysis. An Interdisciplinary Introduction to Univariate & Multivariate Methods, Radius Press, New York.

Levine, N.D., Corliss, J.O., Cos, F.E.G., Deroux, G., Grain, I., Honigberg, B.M., Leedale, G.F., Loeblich, A.R., Lom, J., Lynn, D., Merinfeld, E.G., Page, F.C., Poljansky, G., Sprague, V., Vavra, J. & Wallace, F.G. 1980. A Newly Revised Classification of the Protozoa. *J. Protozool.* 27:37-58.

Tasca, T., G. A. De Carli, Steindel, M., Mattia, R. & Rosset, I. 1999. Modification and adaptation in semi-defined media for cultivation of flagellate *Tetratrichomonas didelphidis* (Trichomonadidae: Trichomonadinae) from the *Didelphis marsupialis*. *Parasitol. al Dia* 23:121-122.