THE CHROMOSOMES OF *RODENTOLEPIS NANA* (SIEBOLD, 1852) SPASSKII, 1954 OBTAINED FROM NATURALLY INFECTED MICE CONVENTIONALLY MAINTAINED IN A BRAZILIAN LABORATORY ANIMAL HOUSE

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Summary:

The karyotype of *Rodentolepis nana* obtained from mice in Rio de Janeiro, Brazil, was described. The diploid chromosome number obtained by the division of embryonic cells was 2n = 12. The first and the third pairs presented subterminal centromeres and the other pairs were all acrocentric. The studied species differed in chromosome morphology when compared to previous description by Mutafova and Gergova (1994) in Bulgaria, suggesting an intraspecific variation.

KEY WORDS : *Rodentolepis nana*, chromosome, karyotype, Cestode, laboratory mice, Brazil.

Résumé : LES CHROMOSOMES DE *RODENTOLEPIS NANA* (SIEBOLD, 1852) SPASSKII, 1954 OBTENUS À PARTIR DE SOURIS DE LABORATOIRE NATURELLEMENT INFECTÉES AU BRÉSIL

Le caryotype de Rodentolepis nana *obtenu à partir de souris de laboratoires à Rio de Janeiro, Brésil, est décrit. Le numéro diploïde des chromosomes obtenus par la division de cellules embryonnaires est 2n = 12. Les première et troisième paires présentent des centromères subterminaux; toutes les autres paires sont acrocentriques. L'espèce étudiée est différente au plan de la morphologie chromosomique de la description préalable de Mutafova et Gergova (1994) en Bulgarie, suggérant une variation intraspécifique.*

MOTS CLÉS : Rodentolepis nana, *chromosome, caryotype, Cestode, souris de laboratoire, Brésil.*

The systematic classification of helminths was based mainly on morphological characteristics. Organisms that show small variations raise doubt about their inclusion in determined genus. The geografic variation and the parasitism of different species are also a factor of doubt in the parasite identification.

The observation of chromosome morphology and diploid number are helpful in helminth identification, however helminths cytogenetic research was slowly developing when compared to other animal Orders. The difficulty in obtaining good preparations is due to the very small size of the chromosomes in this species.

Rodentolepis nana (Siebold, 1852) Spasskii, 1954 is a cestoda of the Family Hymenolepididae, distributed all over the world, found in the small intestine of mice, rats and hamsters, but it may infect humans too, being of importance in public health (Stone & Manwell, 1966; Barbosa, 1975; Tashima & Simoes, 2004). Its prevalence is high in Brazilian laboratory animal houses (Pinto *et al.*, 1994; Gilioli *et al.*, 2000; Bazzano *et al.*, 2002). When the worm weight is very high, the infected animals may show clinical signs and die, especially in the nude mice (Nacional Institutes of Health, 1994). Its occurrence in laboratory animals is not desirable due to its pathogenic potential, interfering on research results involving immunology, haematology, nutrition, enterology and neuroendocrinology (McKay *et al.*, 1990; National Institutes of Health, 1994). The generic name for the cestode *R. nana*, previously referred to either as *Hymenolepis* or *Vampirolepis*, was adopted based on the presence of three tests prepared into two groups by the female gonads in the mature proglottids, a characteristic absent in the last two genus (Czaplinski & Vaucher, 1992). These changes of generic name reflect difficulties in the taxonomy of this helminth. According to Czaplinsky & Vaucher (1992), other important problem is poor knowledge of the variability of many morphological features and of their hierarchy and significance for taxonomy.

Cytogenetic investigations in species of the Family Hymenolepididae have been done by Jones (1945), Jones & Ciordia (1955), Hossain & Jones (1963), Proffitt & Jones (1969), Ward *et al.* (1981), Liu & Lin (1987), Mutafova & Gergova (1994), Spakulova & Casanova (1998) and Casanova *et al.* (2000), with agreement in the uniformity of chromosome number.

Mutafova & Gergova (1994), studying naturally infected mice in Bulgary, described the chromosome of the species *Rodentolepis nana*, with diploid number 2n = 12, where five pairs of chromosomes showed terminal centromere. The last pair sized in third position had the centromere in middling or submiddling location.

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The objective of this paper is to study the chromosome complement of *Rodentolepis nana* obtained from naturally infected conventional laboratory mice bred in CECAL (Laboratory Animals Breeding Center) of the Oswaldo Cruz Institute, Rio de Janeiro, Brazil.

MATERIAL AND METHODS

Seven adult specimens of *R. nana* were obtained from conventionally maintained mice (*Mus musculus*) congenit knockout B6129P2-Nos2^{tm1Lau} bred at CECAL, Oswaldo Cruz Institute, Rio de Janeiro, Brazil (22º 54' S - 43º 12' W), after euthanasia in CO_2 chamber. At necropsy, the small intestine was opened in a Petri dish containing 0.85 % NaCl solution with the aid of a scissor. Seven live helminths were treated with 0.01 % colchicine in saline solution for four hours at room temperature, then transferred to destilled water at 37º C for 40 minutes for hypotony. Thereafter this material was fixed in methanol:acetic acid (3:1) cut, minced and three times centrifuged at 1,000 rpm for 10 minutes. The fixative solution was changed after each centrifugation. The sediment obtained was then suspended in 3-4 ml fixative solution, dropped on slides previously cleaned and air-dried. For chromosome staining, the slides were immersed in 3 % Giemsa solution in phosphate buffer pH 6.8 for five minutes and washed in destilled water.

The best metaphase plates were photographed to organize the karyotype. The development of this study was autorized by the Ethics Committee for the Use of Animals (CEUA/Fiocruz), through the protocol number P0044-00 of approval.

RESULTS

The chromosomes were obtained by the division of embryonic cells (Fig. 1) that were ideal to this cytogenetic study. 30 mitotic metaphasis of *Rodentolepis nana* were analyzed and all the cells had 2n = 12. The karyotype (Fig. 2) was composed of six pairs of homologous chromosomes, organized in order of decreasing size. The first pair was the biggest and yielded subterminal centromere. The second pair was acrocentric, the third also yielded subterminal centromere and the other pairs were all acrocentric.

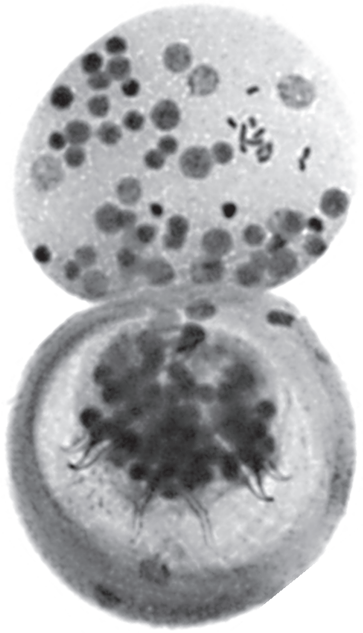

Fig. 1. – Embryos of *R. nana* in different stages of development with a visible metaphase. Bar = 0.01 mm.

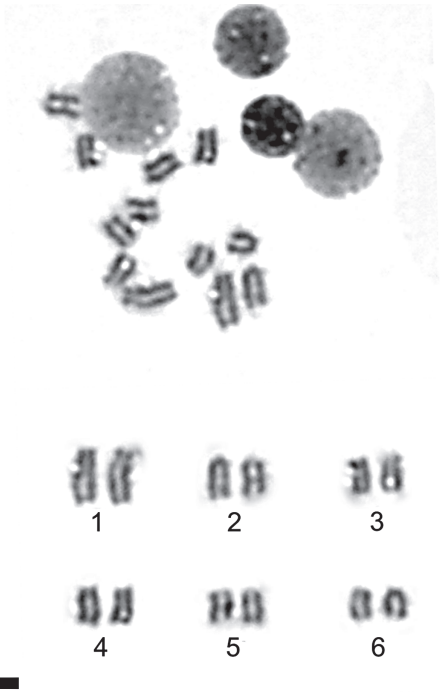

Fig. 2. – Metaphase and karyotype of *R. nana*. Bar = 0.004 mm.

DISCUSSION

Information about the karyotype of hymenolepidids is still restrict. The first study by Jones (1945) provided data about the number of nine species: *Protogynella blarinae* (2n = 10), *Diorchis reynoldsi* (2n = 10), *D. ralli* (2n = 10), *Hymenolepis fraterna* (2n = 10), *H. anthrocephalus* (2n = 12), *H. diminuta* (2n = 12), *H. serpentulus sturni* (2n = 12), *H. serpentulus turdi* (2n = 12), *Aploparaksis* sp. (2n = 12). These data were obtained from cleaved material, where some chromosomes expressed high degree of overlap. After this,

H. fraternal was showed to have 2n = 12 (Jones & Ciordia, 1955). The same chromosome number (2n = 12) was confirmed for *H. diminuta* (Kisner, 1957) and was described for *H. microstoma* (Hossain & Jones, 1963; Proffitt & Jones, 1969) and *H. citelli* (Ward *et al.*, 1981). Because the methods of chromosomes obtainment used by these authors (incision or crushing) do not permit a clear identification of centromeric position, very poor details on chromosome morphology for all those species were reported. Subsequently, a study on *H. diminuta* was accomplished and the chromosomes were morphologically described as two pairs of metacentric chromosomes, three pairs of submetacentric and one acrocentric pair (Liu & Lin, 1987). Nevertheless, Mutafova & Gergova (1994) studied three species of *Hymenolepis*, found only one pair of metacentric chromosomes for *H. diminuta*, being the other five all acrocentric, and they considered this difference as interpopulative. For *H. erinacei*, they described six acrocentric pairs. With regard to *H. nana*, the karyotype was 2n = 12, constituted by five acrocentric pairs, being the third pair a metacentric, different from our results. These literature data confirmed that the species of the genus *Hymenolepis* has a constant chromosomes number of 2n = 12 chromosomes, differing morphologically from each author description. Our findings on *R. nana* karyotype, differing from the results of Mutafova & Gergova (1994) for the same species, suggest an intraspecific variation, possibly due to geographic isolation and successive karyotype reorganization, mainly by pericentric inversions, since the samples were representative of so far regions. More cytological evaluations of the species from the genus *Hymenolepis* and from all cestode groups are necessary to understand the interrelationships among species and their phylogenetic position. We expect that results on helminths cytogenetic studies will reveal many interesting and unexpected aspects of cestoda phylogeny and systematics.

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