

CROSS-INFECTION EXPERIMENTS WITH "ISOSPORA" OF CATS AND DOGS (1)

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SUMMARY: Feces of 34 dogs out of 251 (13.5%) from Guanabara were positive for *Isospora*. From these 19 (7.5%) were *I. rivolta*, 13 (5.2%) were *I. canis* and 2 (0.7%) were *I. bigemina*. "Free-sporocysts" of *I. rivolta* were eliminated by 9 dogs (3.5%). A "Caryospora-like" oocyst was seen once. Cross-infection experiments performed with *Isospora* from dogs and cats failed to produce infection while inoculations of these *Isospora* in their natural hosts succeeded. The results suggest that the species of *Isospora* occurring in cats are different from those of dogs.

THE species of *Isospora* occurring in cats and dogs have similar size and for this reason they have been considered identical (2), (4) (9) (11). Andrews (2) admitted the identity but considered it unusual among mammalian *Coccidia* which generally are strictly host-specific and he cited Böhm (1923) who was the first to have doubts about that identity. The problem of the *Isospora* of bigger size in both hosts was solved when Nemeséri (7), in 1959, separated *I. canis* from *I. felis*. However many questions about *I. rivolta* and *I. bigemina* remain without answer. The present paper reports an investigation about cross-infection experiments with *Isospora* from cats and dogs and its prevalence in dogs from Guanabara.

MATERIAL AND METHODS

Origin of oocysts — Oocysts of *I. canis*, *I. rivolta* and *I. bigemina* of dog origin were obtained from a survey made among 251 dogs mostly from Instituto Estadual de Medicina Veterinária. These oocysts were inoculated into 5 puppies which furnished material for the subsequent experiments. Oocysts of cat origin were obtained from animals used in a previous work(8).

Animals — 25 cats and 33 dogs of various ages (from hours after birth to 2 months) were either purchased or born in the laboratory. Newlyborn puppies or kittens were maintained in an incubator (28° — 30°C) in the first weeks of life and bottle-fed with a mixture of egg-yolk and evaporated milk. Each litter was separated from its mother and kept isolated from other animals during the whole time of the experience. Feeding equipment was all boiled before use. Incubator and cages were always maintained clean. Elder an-

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imals from negative mothers and also isolated from them were used only when considered *Coccidia*-free after being examined for at least 3 weeks.

Inoculations — Fresh samples of feces were mixed with potassium bichromate 2,5% and filtered in a double cheesecloth. A thin layer of the mixture was put in petri dishes for one week at room temperature to sporulate. After the sporulation, if not used on the same day the material was kept in the refrigerator in stoppered tubes. Before inoculation bichromate was removed by three washes with saline. Inoculations were made by means of a plastic tube connected to a syringe which was introduced into the esophagus.

Coproscopies — Feces were collected daily after the first day of inoculation. For the smaller animals it was necessary to rub the anal region with a swab to induce defecation. The examens were made after Shearer's flotation method.

EXPERIMENTS AND RESULTS

1. Natural occurrence of "*Isospora*" in dogs

Oocysts were found in 34 out of 251 dogs (13.5%). Nineteen (7.5%) were positive for *I. rivolta*, 13 (5.2%) for *I. canis* and 2 (0.7%) for *I. bigemina*.

"Free-sporocysts" — Nine dogs (3.5%) eliminated "free-sporocysts" similar in size to those of *I. rivolta* typical oocysts of which were found in 4 of them. Indeed its size was slightly bigger. This difference may be explained by the fact that free-sporocysts are larger than those contained in oocysts because they are free of compression. In one sample there could be seen groups of 2 still attached to each other by remnants of the collapsed oocyst wall. (Fig. I, 7).

"Caryospora-like" oocysts — In a sample positive for *I. canis* only one oocyst was seen with a single sporocyst containing 8 sporozoites. Its size was that of *I. canis* oocysts.

Measures: Fifty oocysts and thirty sporocysts were measured for each species.

I. canis oocysts: 27-42u x 22.5-36u (mean: 34u x 28.4u); sporocysts: 15-22.5u x 15-19.5u (mean: 19.4u x 16.3u).

I. rivolta oocysts: 15-25.5u x 12-22.5u (mean: 21.6u x 18.5u); sporocysts: 12-15u x 7.5-10.5u (mean: 14.4u x 9.4 u).

I. bigemina oocysts: 10.5-15u x 9-13.5u (mean: 12.5u x 10.7 u); sporocysts: 6-6u x 4.5-6u (mean: 6u x 5.3u).

"Free-sporocysts": 15-19.5u x 7.5-13.5u (mean: 16.9u x 10.6u).

2. Dogs inoculated with "*Isospora*" of dog origin

One dog 1 month old received oocysts of *I. canis* and passed oocysts of this *Isospora* in the feces from the 11th day after the inoculation for 3 days. One dog 20 days old received oocysts of *I. rivolta* and was positive in the 5th day and continued eliminating oocysts for 15 days. Four dogs 3-5 days old were inoculated with *I. canis* and *I. bigemina* and 3 of them became positive for *I. canis* 14 days later and eliminated oocysts for 16 days. None of these dogs eliminated oocysts of *I. bigemina*. Three dogs 20 days old received oocysts of *I. canis*, *I. rivolta* and *I. bigemina* and eliminated oocysts of these *Isospora* 5 days later during 15 days. One dog 12 days old received "free-sporocysts" of *I. rivolta* and was always negative.

3. Dogs inoculated with "*Isospora*" of cat origin

Twenty-one dogs 2-30 days old were orally inoculated with oocysts of *I. felis* and *I. rivolta*; two dogs age 1 month received oocysts of *I. rivolta* only. All coproscopies of these animals were negative. The period of examinations varied from 10 days to 6 months.

4. Cats inoculated with "*Isospora*" of cat origin

Two cats age 1 month received oocysts of *I. felis* and *I. rivolta* and were positive for these *Isospora* 4 days after the inoculation, passing oocysts for 8 days.

5. Cats inoculated with "*Isospora*" of dog origin

Thirteen cats 1-9 days old received oocysts of *I. rivolta*; two cats 4 days old

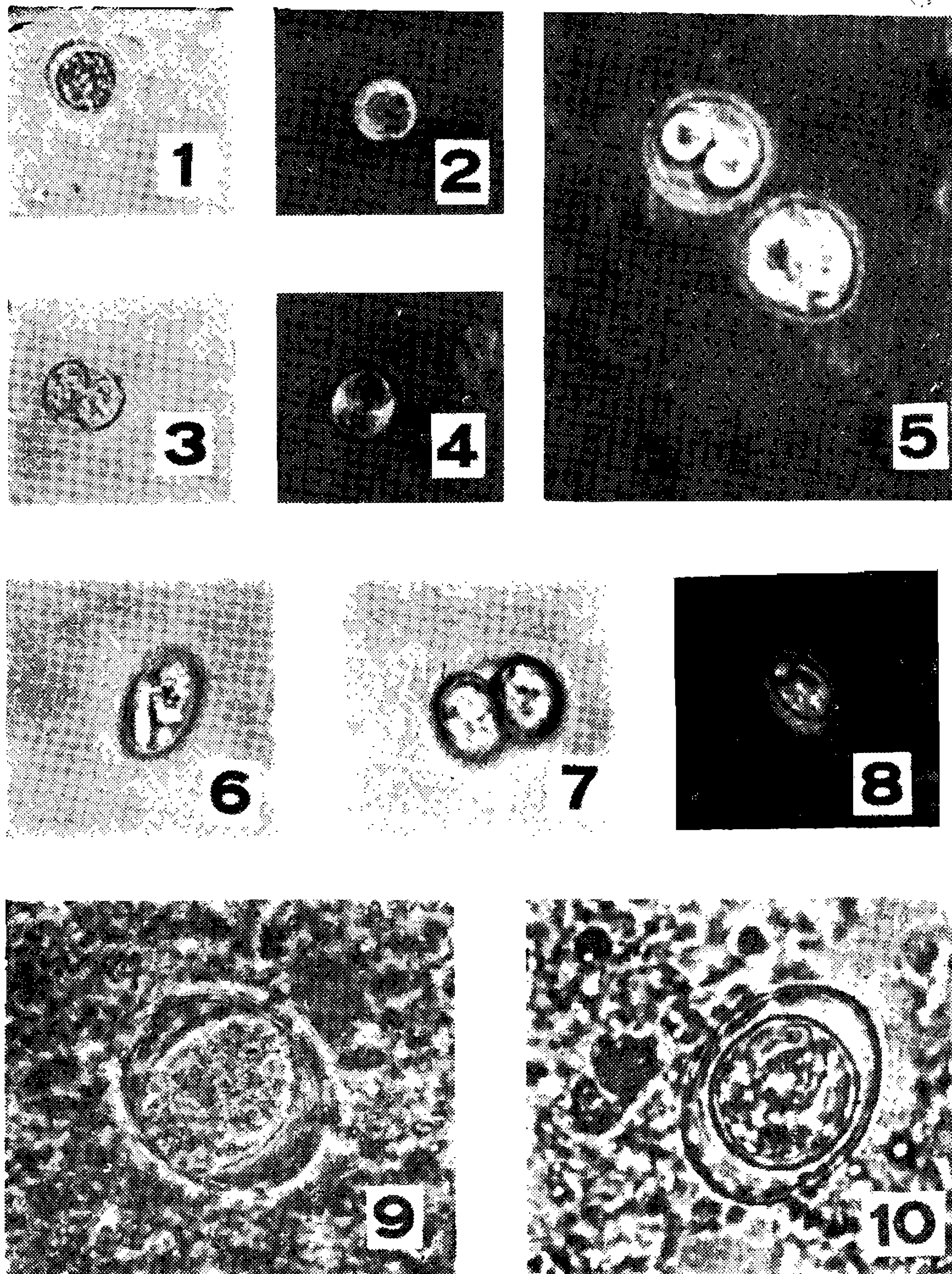


Fig. 1 — *Isospora* of dog origin. 1-4 *I. bigemina*. 1. Unsporulated oocyst; 2. Unsporulated oocyst (Phase contrast microscopy); 3. Sporulated oocyst; 4. Sporulated oocyst (Phase contrast microscopy). 5-8 *I. rivolta*. 5. Sporulated oocysts; 6. Free-sporocysts showing sporozoites and residuum; 7. Sporocysts not completely free; 8. Free-sporocysts (Phase-contrast microscopy). 9. *I. canis* — unsporulated oocyst with an ovoid sporont preparing to divide. 10. Abnormal oocyst "Caryospora-like" (*I. canis?*). Obj. 40x; oc. 10x.

received oocysts of *I. rivolta* and *I. canis*, and two cats 2 days old received oocysts of *I. canis* and *I. bigemina*. None of these seventeen cats ever eliminated oocysts.

Eight kittens were obtained by hysterotomy made in two cats. It was difficult to rear them and as death occurred very soon only one of 5 days old was inoculated

with *I. canis* and *I. rivolta* with a negative result.

The description of the endogenous cycles of *I. felis* and *I. rivolta* in naturally infected cats has already been done in a previous work⁽⁸⁾. A remarkable fact was the finding of a macrogametocyte in the liver of one of those cats with *I. felis* (fig. II, 5).

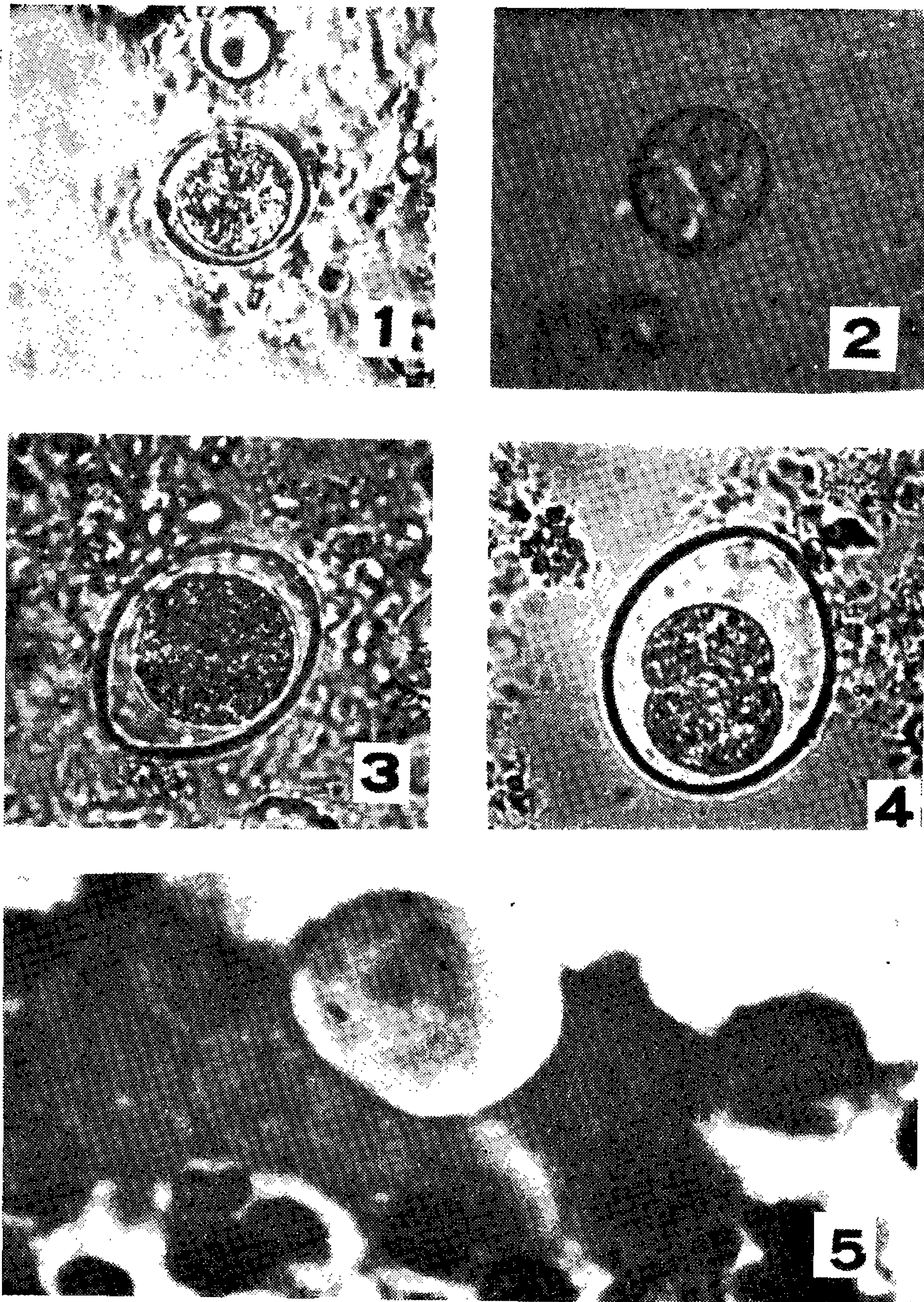


Fig. II -- *Isospora* of cat origin. 1 and 2 *I. rivolta*. 1. Unsporulated oocysts; 2. Sporulated oocyst showing sporocysts and sporozoites within them. 3 and 4 *I. felis*; 3. Unsporulated oocyst; 4. Sporulating oocyst — sporont beginning cleavage; (Obj. 40x; oc. 10x) 5. Macrogametocyte of *I. felis* in a section of liver. (Obj. 100x; oc. 10x, Hematoxylin-eosin).

DISCUSSION

The occurrence of *Isospora* in dogs in Brazil, reported by various authors (1926-1968) was summarized by Rocha & Lopes⁽⁹⁾. The prevalence obtained by us in Guanabara is 13.5% for all the species. Concerning *I. canis*

we have found a much higher percentage than Amaral & cols.⁽¹⁾ which found only 0.8% in S. Paulo.

All attempts made to transmit *Isospora* from dogs to cats or vice-versa failed although we inoculated animals of different ages. To insure that oocysts used were viable they

were inoculated either freshly collected or after being kept in the refrigerator for 2 months or even more and in both cases they succeeded in infecting animals when their homologous hosts were inoculated.

Some authors in the past like Wenyon(11) and Lee(4) and more recently Rocha & Lopes(9) stated to have accomplished with success the cross-infection of *Isospora* of cats and dogs. However in all those cases the history of the animals was poorly known or they were insufficiently examined prior to inoculation. Some of our purchased animals which were negative for 20 days were then found to be positive and had to be excluded from the experience. Marht(6), Shah (10) and Nery-Guimarães & Lage(8) also failed in cross-infecting cats and dogs.

The separation of *I. felis* from *I. canis* made by Nemeséri (1959) is a fact accepted by all. Probably *I. rivolta* of dog origin is also a different species from *I. rivolta* of cats.

The finding of an extra-intestinal evolutionary form of the endogenous cycle (macrogametocyte) in natural

infection of *I. felis* corroborates the findings of Dubey & Frenkel(3) as the occurrence of extra-intestinal evolution in *Isospora* in experimental infection.

SUMÁRIO

Dos 251 cães da Guanabara examinados 34 (13,5%) foram positivos para *Isospora*. Desses, 19 (7,5%) foram positivos para *I. rivolta*, 13 (5,2%) para *L. canis* e 2 (0,7%) para *I. bigemina*. "Esporocistos livres" de *I. rivolta* foram eliminados por 9 cães (3,5%). Foi visto uma vez um oocisto do tipo "Caryospora". Infecções cruzadas realizadas com *Isospora* de cães e gatos tiveram sempre resultados negativos, enquanto que resultados positivos foram obtidos quando oocistos das mesmas espécies, submetidos às mesmas condições, eram inoculados nos seus hospedeiros naturais. Os resultados sugerem que as espécies de *Isospora* que ocorrem nos gatos são diferentes das do cão.

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