On a new protozoan parasite of rabbits+

2nd Preliminary note

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In a report (1) to the S. Paulo Scientific Society on July 16th, 1908 (see Report Book), I presented a new rabbit disease whose anatomical lesions resemble human Kala-Azar. In fact, there were parasitic cells resembling *Leishmania*, with the only relevant difference being that the centromere was undetectable under Romanowky's staining.

The first spontaneous cases recorded in my laboratory occurred last year, in the first days of June. Many others were observed in July and August; the incidence decreased thereafter, and, after October there was a long delay before a patently new case occurred.

I will now describe some new observations.

In January of the current year, the previously reported lesions were found in a new case, in a rabbit that died spontaneously. In this case, characteristic lesions were prominent in the lungs, sparse in the spleen, and very rare in the liver. In fact, very few characteristic kidney-shaped cells were observed under microscopy (see fig. 5). However, the cells filled with chromatin blocks that were described in the first report were very easy-to-find, though not abundant. Identical lesions with identical localization were found again in two experimental rabbit series infected by inoculation. A third inoculation series gave completely negative results.

These occurrences may be related to seasonal conditions influencing the complete development cycle.

In a new group of cases occurring in the same period last year, microscopic examination revealed the same anatomic and parasitic situation. I am of the opinion that seasonal variation must be taken into consideration in a transmission study, as constant transmission has never been possible.

Regarding the parasites' aspect, in these new cases, both cell-free amoeboid parasites and fusiform parasites with flagella, the latter missing chromatin, were observed in considerably large numbers.

A large pyriform body was observed in a liver-derived smear; it had a diameter of 3 to 4 red blood cells and had several chromatin blocks, three of which were located transversally at the base. Another group was detected near the apex, with a flagellum-like thread 5-6 μm in length.

I have never observed active movement in fresh wet preparations.

Besides cystic forms with an undetermined number of free or intra-cellular parasites (see figs. 1 and 2) some other parasites, really rare, consistently showed only 8 elements (see figs. 3 and 4), showing certain similarities with many microsporides as I described in previous papers.

I would like to remark that parasite reproduction shows two modalities: the first one is longitudinal bipartition (see fig. 5), and the second one is multiple endocellular partition (schizogony).

During experimental transmission, a great pathogenic potential was evidenced, presumably due to the presence of toxins.

Healthy rabbits that were subcutaneously injected with emulsified organs of infected animals sometimes died before a characteristic lesion on targeted organs could be detected. In these cases, the parasitic forms were scarce, always amoeboid-shaped, and mostly located in mononuclear cells or adhering to the nucleus of the host cell, an aspect that reveals similarities with the kidney-shaped cells that appear to be in the growth/reproductive phase of the cell cycle. Amoeboid parasites found later lacked any distinctive characteristics; they were still visible as sparse alveolar cells on another cell body whose cytoplasm was progressively invaded and whose nucleus changed position due to the resulting pressure. In this phase, parasite chromatin appeared as a lump a little wider than normal, and was poorly stainable with Giemsa.

Later, the chromatin became paler and more diffuse; at the end, it was dispersed in a very small and barely visible granule in the cytoplasm. On the 6th experimental day, the first clinical symptoms of endogenous multiplication were observed. Death occurred between the 12th and the 15th days after inoculation; in one case it occurred earlier, on the 9th day.

Other animal species, mainly mice, one of which died within a few days, showed the characteristic lesions of the disease, mainly in the lungs. Parasite numbers in those animals were found to be scarce.

A dog injected with emulsified tissues extracted from a rabbit showing a high number of parasites suffered from bloody diarrhoea in the first few days, and 2 months later was suffering from severe progressive wasting disease and loss of sight due to ocular turbidity. The animal seemed near death: it could neither move nor eat, but it later resumed feeding and recovered strength. It was sacrificed at that moment before complete recovery to study the parasite cycle. No parasite and no lesions were found.

Surprisingly, rabbit parasites can reproduce in birds, as shown last year with two sparrows (*Zonotrichia pilea-*

+ Kindly translated by Wilma Buffolano, from the original Italian paper published in *Rev Soc Sci S Paulo 4*: 76-79, 1909.

ta) that died 5 days after subcutaneous injection of two spleen drops extracted from an infected rabbit. In these birds, lesions were not evident and typical cells were few.

Several small birds of the *Euphonia* genus, injected according to the protocol described above, died on days 8 and 11; the liver and spleen were already very enlarged and filled (fig. 6) with an enormous number of typical parasite cells, some of them present in cardiac blood inside the red blood cells.

Recently, Dr. Carini has confirmed my reports on rabbits and obtained abundant parasite reproduction in the organs of doves, in which species I have identified systematic serial transmission of the disease. Subcutaneously or intra-muscularly injected rabbit parasites killed these birds on days 11 to 15 after inoculation. A great amount of parasites was found in all internal organs, mainly in the liver; furthermore, they lacked macroscopic alterations. In one of these cases, relatively numerous (8 parasites) typical forms were observed.

In conclusion, we are witnessing an interesting new parasite, part of a protozoan group different than any previously described genus, based on both morphological and pathogenic characteristics.

Shortly after my discovery, another protozoan morphologically identical to that from the rabbits was found in an African rodent (*Ctenodactylus gondii*) in Tunisia, by Nicolle and Manceaux (2). I had the opportunity to study this parasite in slide preparations kindly provided by Nicolle; it is different from mine, as it lacks endogenous replication.

Undoubtedly, these two species must be classified under the same genus and, as Nicolle proposed the name *Toxoplasma* (pointing to the arched shape of the cells), I will adopt the provisory name of *T. cuniculi* for the rabbit parasite.

1- Rev. da Soc. Scient. De S. Paulo 3:109-112, 1908

2- *Com. Rend de l'Ac. De Sc. 26 oct 1908 et 8 fevrier 1909; Arch de l' Inst. Pasteur de Tunis fasc. II Mai 1909.*

Legend to Figures

Fig. I-II: Lump of free *Tox.cuniculi*, undetermined number of corpuscles
Fig. I observation of fresh material
Fig. II followed by Giemsa staining

Fig. III Eight corpuscles of *T. cuniculi* not yet fully individualized, confined to a single mononuclear cell

Fig. IV: Mass free of *Tox cun*. eight almost completely differentiated corpuscles

Fig. V: Characteristic corpuscles of free *Tox. Cun.* one of them in longitudinal bipartition.

Fig. VI-VII: Experimental replication in spleen of *Euphonia purple* Linn. Image Magnification: ob. Ap. Zeiss 2mm I. oc. Comp.4