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TRYPANOSOMA INGENS IN THE MOUSE DEER.
(TRAGULUS JAVANICUS).

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EARLY in October 1911 three mouse deer (*tragulus javanicus*) were brought from Java to the Royal Zoological Society's Gardens at Sydney. They had been captured only a few weeks prior to their arrival and were all apparently in good health when received.

One of the animals died on 25th October 1911, about two weeks after arrival. Its attendant had not observed any marked signs of ill health before death.

Post-mortem examination held next morning did not reveal any gross lesions, but there was a slight general œdematous condition of the tissues. Examination of the blood revealed the presence of fairly numerous micro-filariæ, and what were probably the adult filariæ were found between the layers of the mesentery and in the connective tissue at the apex of the pericardium, at the point of its connection with the sternum. In the blood, in addition to the micro-filariæ, one could discern occasionally organisms, which in size and outline were not greatly unlike the smaller micro-filariæ. In details, so far as such could be made out, however, they differed considerably, but on account of *post-mortem* changes the parasites did not stain well; consequently there was doubt as to their exact nature, although occasionally, where their structure was not entirely confused or disintegrated, one could see that they were more like

gigantic trypanosomes, which, indeed, they were subsequently ascertained to be.

The latter parasites, although they could not be termed numerous, were, nevertheless, to be found in any blood smear, or that from any of the organs. Blood lesions were present, but not to any marked degree.

The second mouse deer became visibly ill a few days before its death, which occurred on 29th October 1911.

Gross lesions were absent upon *post-mortem* examination, as in the first animal. A slightly cedematous condition of the tissues generally was observed. Adult filariæ were numerous present in the situations noted in the previous mouse deer. Micro-filariæ were scantily present in the blood from the large vessels, but more numerous in the lungs, liver, and kidneys. Blood lesions were not very pronounced. The large trypanosomes were present in the blood in the vessels and organs, several parasites being found in every film. Fortunately in this case they were alive and fairly vigorous. The lashing movements executed by the parasite were of a very leisurely character, by no means so energetic or rapid as, for instance, that of *trypanosoma evansi*. There was also a distinct movement of translation, but this was not extensive, the organism remaining in the field of the microscope for some considerable period. In the hanging drop preparations, on account of the great size of the trypanosomes, if only casually examined they could be readily confounded with the smaller micro-filariæ, which were also rather numerous present in the blood.

Stained blood films showed that the flagellated organisms were identical with those found in the first animal.

On account of the discovery of this unidentified parasite, which at that time was believed to be a new species, in the two dead mouse deer, it was decided to examine the blood of the remaining animal at intervals, in order to decide whether the new organism was present at all in the survivor, and, if it were, to ascertain whether it increased or decreased in numbers, and to gain other data.

The animal at that time was apparently in good health. External appearances, however, in wild animals just placed in captivity are not of much assistance unless the animal be very ill indeed. Blood films were prepared from the ear at varying intervals and at different times during the day and night for about six months, with the result that the trypanosome was found to be present on every occasion. One cannot, however, say that they were numerous. On an average about two trypanosomes were found in every three drops of blood. Their numbers did not vary greatly during the period of examination except just prior to death, when there was a moderate increase, from about five to ten organisms being then present in a blood film.

Several rabbits and guinea-pigs were inoculated with fresh blood from this animal, but in each case with a negative result. No blood counts were attempted, because the normal blood count of the mouse deer is unknown, and all my animals were diseased. A blood count in such case would only have been of value if it had been very low.

At this period I was unable to identify the organism, and con-

sequently believed it to be a new species because of its enormous size and from its staining reactions. (I had not at that time seen Sir D. Bruce's article on *trypanosoma ingens*, nor did other literature then at my disposal assist me greatly). Indeed, in some instances, where the flagellum was entirely hidden and the parasites badly stained and huddled up into a shapeless mass, as not infrequently was the case, they had some superficial resemblance to the smaller micro-filariæ, although this was only superficial, as they differed in important details.

Early in April 1912 the third mouse deer began to show unmistakable signs of ill-health. At the same time the number of trypanosomes in the peripheral circulation slightly increased. The animal died on 21st April. Again gross lesions, except a moderately dropsical condition of the tissues generally, were conspicuous by their absence upon *post-mortem* examination. The immense trypanosomes were present in moderate numbers in the blood stream, but scanty in the organs. Micro-filariæ were found in the vessels, lungs, liver, kidneys, and spleen. Changes in the blood itself, although present, were not very marked.

In order to settle the point of the new parasite's identity, I sent specimens and drawings to Professor F. Mesnil, of Paris, for his opinion early in April 1912, and I have to express my appreciation of his courtesy in examining and identifying the trypanosome.

Professor Mesnil states he is of opinion that the trypanosome is probably *trypanosoma ingens*, discovered by Bruce in Uganda, and described by him in the *Proceedings of the Royal Society*, Vol. 81 B. He further states that Rodhain and his colleagues record the discovery of a trypanosome in the chevrotian on the Congo, West Africa, in the *Bulletin Soc. Path. Exotique* for May 1912. (The tragulidae are also known as chevrotians). Unfortunately I have been unable to peruse Rodhain's article, and therefore am unable to compare his trypanosome with that discovered by me, or to say whether they are identical or not.

Since my attention was directed to it, I have, however, been able to consult Bruce's article on the *trypanosoma ingens*, and to compare his description and drawings with the trypanosome from the mouse deer.

Bruce's trypanosome (*trypanosoma ingens*) was discovered in the blood of a reed-buck, then in a bush-buck, and finally in an ox, in Uganda. Alive, in fresh preparations, Bruce states, the parasite has a slow and deliberate movement. When stained, the trypanosome measured up to 122 microns, and even then it appeared capable of stretching out to a greater length. His other measurements given were 72, 77, 88, and 82 microns. The breadth was from 7-10 microns. The micro-nucleus was round and about a micron in diameter, lying posterior to, and quite close to, the nucleus. From it a well-marked but narrow undulating membrane arose, ending at the anterior extremity in a free flagellum. Nucleus oval and lying across the body. Situation nearer the posterior than the anterior end, and stained a pale pink. Body markedly granular behind the nucleus, while in front of the nucleus the structure described as myonemes was particularly well marked.

The following are the more minute measurements of Bruce's *trypanosoma ingens*:—

	<i>Microns.</i>
Posterior end to micro-nucleus	18
Micro-nucleus to macro-nucleus	4
Nucleus—Long diameter 8μ ; short diameter	4
Nucleus to anterior end	40
Free flagellum	17
TOTAL	83

For comparison with the above, the following are the measurements of the largest and smallest of eight trypanosomes from the mouse deer, and also the average measurements of the eight, adopting the same nomenclature as Bruce:—

	<i>Largest.</i>	<i>Smallest.</i>	<i>Average.</i>
Posterior end to micro-nucleus	46·4	22·4	34·5
Micro-nucleus to macro-nucleus	4·8	5·6	4·2
Macro-nucleus—long diameter	6·5	5·5	5·3
" " short diameter	4·8	6·0	4·0
" " to anterior end	50·0	26·0	37·0
Free flagellum	24·0	10·0	13·5
TOTAL LENGTH (microns).	130·0	70·0	93·2

Breadth—maximum, 8μ ; minimum, $6·5\mu$; average, $6·6\mu$.

Diameter of micro-nucleus, about 1μ .

Total individual lengths of eight trypanosomes, 70, 118·8, 80, 72, 86·7, 75·5, 130, and 122·3 microns. From the foregoing it is seen that the measurements differ in some respects from those of Bruce's *trypanosoma ingens*.

In staining reactions (with Giemsa's stain) there were also some divergences from Bruce's description and drawings. In the trypanosome from the mouse deer the kineto-nucleus, although posterior to the tropho-nucleus, is not invariably situated close to the latter. Indeed, in several instances there is a considerable interval; the shortest distance was $1·5\mu$, and the greatest $7·5\mu$. The kineto-nucleus stains a bright pink, but is not always round, sometimes it is oval.

In position and staining reactions the tropho-nucleus is similar to that of *trypanosoma ingens*. It is oval and stretches right across the body, its long diameter being at right angles to the length of the body. It stains a very pale pink, and is situated about the centre of the body, but not infrequently nearer the posterior than the anterior extremity. The posterior end is sharply pointed. The anterior end terminates in a free flagellum.

The body substance stains a more or less deep purple, and is markedly granular in some instances. In others it is fairly homogeneous. A clear area of varying extent usually surrounds the kineto-nucleus. There is, however, no striation of the body substance anterior to the tropho-nucleus, as described and figured by Bruce.

In wet films the trypanosomes remained alive about thirty-six hours at ordinary temperature. The lashing movements were of a very deliberate nature, more like those of a micro-filaria. The movements from place to place were slow and not extensive.

The trypanosomes found in the organs varied considerably in size,

Plate II.

being, as a rule, larger than those in the general circulation. The larger parasites were frequently so huddled up that it was difficult or impossible to measure them accurately. There was also a great range in the measurements between similar points in different individuals.

Taking all things into consideration, in spite of the minor differences between the two, I concur with Professor Mesnil in considering that the trypanosome under discussion is probably identical with that discovered by Bruce in Uganda, and named by him *trypanosoma ingens*. As before mentioned, I am not in a position to say whether it is identical with Rodhain's trypanosome from the chevrotian.

In connection with the discovery of a new trypanosome, a matter of great economic importance is its pathogenic or non-pathogenic character. Bruce advances no opinion in this direction in connection with his discovery, and the limitations imposed upon one working in this country prevent me from throwing any definite light upon the subject. I have inoculated rabbits and guinea-pigs with fresh blood containing mouse deer trypanosomes, but with negative results. Such restricted experiments, however, when negative, are of little value. If this trypanosome be identical with *trypanosoma ingens*, then it should be inoculable into cattle. It may of course be non-pathogenic to them, like *trypanosoma theileri*, but it would be well to await further evidence before arriving at any conclusion. In the case of the mouse deer the evidence, although only circumstantial, appears to indicate that in that animal at least the trypanosome may be pathogenic. The animals in question were, it is true, also affected with filariasis, but none to such an extent as to account for death. The first animal showed a moderate piroplasmiasis, but this was probably a recrudescence of that disease, such as frequently occurs in animals recovered from piroplasmiasis when their vitality is greatly lowered by any means. The piroplasmiasis was not sufficient to account for death. The other two animals showed nothing but trypanosomiasis and filariasis. The *post-mortem* appearances also were just those so frequently associated with the majority of trypanosome infections. There is therefore reason for suspecting that the rôle of *trypanosoma ingens* in the mouse deer at least is not entirely a harmless one.

One need not labour the point, however, that further information is desirable concerning this parasite. It is probable that *trypanosoma ingens* is pretty wide spread in Java, and also that it will be found to exist in other localities in that part of Asia. No doubt workers in these places will be able to throw more light on the subject.

DESCRIPTION OF PLATE II.

Figs. 1, 2, 3, 4. *Trypanosoma ingens* from general circulation of mouse deer, drawn with Abbé Camera Lucida. Zeiss Comp. Ocular No. 8. Apochrom. Obj. 1.5 mm. Tube length 160 mm.

Fig. 5. *Trypanosoma evansi* drawn to same scale as above for comparison.

Red blood cells of mouse deer also to scale.

The figures of *trypanosoma ingens* from the mouse deer shown in the plate are below the average length, but they were selected on account of the grosser morphological characters being well demonstrated.