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Isolation of Trypanosomes of the *T. brucei* Group from Lion

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After seven cases of human sleeping sickness occurred in the Serengeti National Park of Tanzania between 1964 and 1966 (TANZANIA NATIONAL PARKS, 1966) a survey on the infection of game animals with trypanosomes was carried out at the Kirawira Veterinary Field Station, located adjacent to the Park in the Musoma District. In the course of this survey, trypanosomes of the *T. brucei* group have been isolated from wildebeest, topi, hartebeest, waterbuck, impala and warthog (BAKER, SACHS and LAUFER, 1967). In addition to these, two lions (*Panthera leo massaicus*) were recently found to be infected with *T. brucei*, apparently the first recorded instances for this carnivore. The only published records of trypanosome infection in lions of which we are aware, are those of WECK (1914) in which the species of trypanosome was not identified, and BAKER (1960) and BAKER et al. (1967), of *T. congolense*.

Material, Methods and Results

In August and December, 1966, the blood of two lions was tested for trypanosomes by inoculating it into white rats. The case history of each animal is given below.

Case Nr. 1

On August 9th, a very emaciated and lethargic female lion cub, about 14 months old, was observed with several other lions near the upper Mbalangeti River, about 16 miles southwest of Seronera in the Serengeti National Park. This animal was injected with succinylcholine chloride by dart syringe in the flank. Blood from the jugular vein of the tranquillized lion was then withdrawn into a sterile syringe containing a little heparin and

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immediately inoculated intraperitoneally into two white rats at a dose of 1 cc and 2 cc, respectively. At the same time, a thick blood smear of the fresh lion blood was prepared and stained by MACLENNAN'S (1957) method; the microscopical examination of the blood slide under $\times 100$ oil-immersion objective was negative.

One rat showed a trypanosome infection four days after being inoculated with lion blood: about 15 trypanosomes were seen per 50 microscopic fields of view when examining the fresh blood with a $\times 40$ objective and a $\times 8$ ocular. The other rat was still negative at this time, but showed a heavy parasitaemia when examined again seven days after inoculation.

Thin blood films prepared from the infected rats and stained with Giemsa after fixing them with methanol, showed heavy infection with trypanosomes of the *T. brucei* type. The parasites were polymorphic with a long, free flagellum in the slender forms and a marginal kinetoplast.

After passing the *T. brucei* strain from the infected rats into two new ones, the former were sent to the London School of Hygiene and Tropical Medicine for storage of the strains at -70°C . The subinoculated rats showed heavy parasitaemia four days after injection, and both died after 20 days. Examination of the blood at 3-days intervals showed that the parasitaemia increased steadily until death.

Case Nr. 2

On December 9th, an adult lioness in seeming good condition died during an ear tagging operation while tranquillized with succinylcholine chloride near the upper Mbalangeti River, Serengeti National Park. The animal was transported to the Kirawira Field Station where, $3\frac{1}{2}$ hours after death, two white rats were inoculated with 1.5 cc and 2.5 cc, respectively, of the lion blood from the jugular vein. The blood was by that time very blackish and thick but not coagulated. The thick smear prepared from this blood again was negative for trypanosomes during the microscopic examination.

Both rats showed a very high parasitaemia when examined six days after inoculation. One rat was sent to London for further research, the other died after 30 days. This strain also exhibited the typical morphology of the *T. brucei* group, and characteristically proved to be very pathogenic for white rats. When passed into two further rats (which received 2 and 1 drop, respectively, of the infected rat blood into the peritoneal cavity), the former rat became positive 3 days after inoculation: 2 trypanosomes

could be seen per 50 fields of fresh blood examined with $\times 40$ objective and $\times 8$ ocular. The latter became positive 4 days after inoculation: 15 trypanosomes per 50 fields of view. The following day, the blood of both rats swarmed with trypanosomes: about 30 trypanosomes per each field of view. Again, one of the infected rats was sent to London for further research. The parasitaemia of the other went down slightly on the sixth day of infection (about 10 trypanosomes per each field of view), only to increase steadily after that, causing death of the rat 22 days after inoculation.

Summary and Discussion

Trypanosoma brucei was isolated from two lions by means of blood inoculation into white rats. The rats became positive 4–6 days after intraperitoneal injection of 1.0 cc–2.5 cc of lion blood. The trypanosomes were highly pathogenic to rats, causing death within 30 days.

The infection of lion with *T. brucei* is of some interest because of the recent outbreaks of human sleeping sickness in the Serengeti National Park. However, before the lion can be implicated as a reservoir of human sleeping sickness, it is necessary to determine if the trypanosomes of the *T. brucei* group isolated from these cats are infective to man. This can only be done by inoculating the suspicious strain into human volunteers.

It is of further interest to note that the incidence of trypanosome infection in the Serengeti lions seems to be high. Microscopic examination of thick blood films from 32 lions, stained by MACLENNAN'S (1957) method, revealed that 22 (68.8%) of these were positive for trypanosomes of an as yet unidentified species.

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Zusammenfassung

Von zwei Löwen der Serengetisteppe in Tanzania wurden durch Blutübertragung auf weiße Ratten Trypanosomen isoliert, die morphologisch zur *T. brucei*-Gruppe gehören und sich als sehr pathogen für Ratten erwiesen. Dies ist der erste Bericht über das Vorkommen von *T. brucei* beim afrikanischen Löwen.

Die Löwen der Serengeti scheinen in hohem Grade mit Trypanosomen infiziert zu sein. Bei der mikroskopischen Untersuchung dicker Blutaussstriche von 32 Löwen wurden bei 22 (68,8%) der Raubtiere Trypanosomen gefunden, die allerdings noch nicht näher bestimmt worden sind.

Die Befunde gewinnen insofern an Bedeutung, als während der letzten zwei Jahre in der wildreichen Serengeti, die nur von wenigen Menschen bewohnt wird, sieben Fälle von Schlafkrankheit aufgetreten sind und an das Vorhandensein eines Reservoirs für menschenpathogene Trypanosomen bei Wildtieren gedacht werden muß.

Résumé

Par passage sanguin sur rats blancs, il nous fut possible d'isoler des trypanosomes à partir de deux lions de la steppe de la Sérengeti. Morphologiquement, ces trypanosomes appartiennent au groupe *T. brucei*. Ils se montrèrent très pathogènes pour les rats. C'est la première fois que *T. brucei* est signalé chez le lion africain.

Les lions de la Sérengeti semblent être infectés à un haut degré par les trypanosomes. L'analyse microscopique de gouttes épaisses du sang de 32 lions a révélé que 22 (68,8 %) hébergeaient des trypanosomes. Toutefois, ceux-ci ne sont pas encore déterminés avec précision.

Ces trouvailles sont importantes car, lors des deux dernières années, 7 cas de maladie du sommeil furent mentionnés dans la Sérengeti, c'est-à-dire dans une région riche en gibier, mais peu habitée. Aussi doit-on penser au rôle de réservoir pour les trypanosomes pathogènes humains que peuvent y jouer les animaux sauvages.