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**Autor:** Woo, P.T.K. / Kauffmann, M.
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# The Haematocrit Centrifuge Technique for the Detection of Low Virulent Strains of Trypanosomes of the *Trypanosoma congolense* Sub-Group

P. T. K. Woo [1] and M. Kauffmann [2]

Trypanosomes of the *T. vivax* and *T. congolense* sub-groups are the most important causative organisms of animal trypanosomiasis in Africa. Numerous surveys have been conducted to determine the incidence of these trypanosomes in cattle and wild animals in various parts of the continent. The most commonly used and considered most reliable techniques for the determination of the incidence of *T. congolense* infections are blood inoculations into small laboratory rodents and by thick smears. Both these techniques are often used simultaneously because it is generally accepted that some strains of *T. congolense* may not be infective, or do not produce a detectable infection (as determined by wet preparation) in mice or rats. In a survey on the incidence of trypanosomiasis in migrating cattle in Nigeria, Killick-Kendrick & Godfrey (1963) found that 9 (13 %) out of 66 infections were missed by animal inoculations, but detected by thick smears.

Transient trypanosome infections in mice, rats, and guinea pigs due to freshly isolated strains of *T. congolense* sub-group have been recorded (Dutton, Todd & Kinghorn 1907) and strains with long incubation periods in rats are known (Killick-Kendrick & Godfrey 1963).

The haematocrit centrifuge technique (Woo 1969) has been used successfully for the parasitological diagnosis of human trypanosomiasis under laboratory conditions. In a recent study (Woo 1971) it has been shown that this technique can be adapted for the diagnosis of the infections under field conditions. The purpose of the present study is to investigate the possibility of using this centrifuge technique to (1) confirm the continued presence of trypanosomes in mice which had shown a transient infection, and (2) detect sub-patent infections in mice which had been inoculated with blood from wild animals.

## Materials and Methods

Blood from various wild animals was inoculated intraperitoneally into mice during the E.A.T.R.O. and S.T.I. Trypanosomiasis survey at the Serengeti National Park, Tanzania (October/November 1970). The blood of the mice was examined by wet preparation every other day during the first 25 days. After 24–26 days, mice that had shown a "transient" infection or were "negative" during this period were then examined by the haematocrit centrifuge technique (H.C.T.). Those mice which were positive by the H.C.T. were isolated and their blood checked by wet preparation (at least 40 microscopic fields using 10× objective) every other day and by H.C.T. every 7th day. Two thick blood smears were made from each mouse on the 75th day before the mice were destroyed. The smears were then air dried and stained in Giemsa's stain. In most cases the whole thick smear

[1] F.A.O. André Mayer Research Fellow, Canadian Medical Research Council Research Fellow, Department of Veterinary Microbiology and Immunology, University of Guelph, Guelph, Ontario, Canada.

[2] Swiss Tropical Institute, Socinstrasse 57, 4051 Basle, Switzerland.

(about 3 cm. radius) was examined under high dry (8 × eye piece and 40 × objective) for trypanosomes. Trypanosomes were frozen and stored in liquid nitrogen at the E.A.T.R.O., Tororo, Uganda.

## Results

By using the H.C.T., trypanosomes belonging to the *T. congolense* sub-group were demonstrated in many of the mice that were supposed to be negative by wet preparation and by thick smear. These mice had been inoculated with blood from 1 hyena (103) and 1 lion (105). In addition, it has been shown that trypanosomes were continuously present in the blood of mice that had shown a transient infection by wet preparation. These mice had been inoculated with blood from 2 other lions (104, 106).

Identification of the trypanosomes in these mice has been based on stained specimens from both thick and thin smears (in cases where a transient infection was encountered) and/or by the characteristic movements of the trypanosomes in the centrifuged capillary tubes.

1. Hyena 103; adult female: At no time were trypanosomes seen in wet preparations or thick smears from all 7 mice which were inoculated with blood from this animal.

On the 26th day, the blood of all 7 mice was examined by the haematocrit centrifuge technique. One of the 7 mice was found to be infected. When the mice were re-examined 7 days later an additional 3 mice were found to be infected. The infection in all 4 mice was low (1–3 trypanosomes per 0.06 ml. of tail blood) and all infected mice remained infected till the 75th day when they were destroyed.

2. Lion 104; adult male: Two of 10 mice were found to be infected with trypanosomes of the *T. brucei* sub-group; the trypanosomes from one of the 2 mice were frozen (E.A.T.R.O. 1811). One of the remaining 8 mice died on the 9th day; no trypanosomes were seen by wet preparations until the 56th day when a low grade *T. congolense* sub-group infection (1–3 trypanosomes per 10 microscopic fields) was detected in one of the 7 mice. This infection was detectable for 4 days after which all the mice were again "negative" by wet preparation and thick smears. The mice were destroyed on the 75th day.

Using the H.C.T., trypanosomes belonging to the *T. congolense* sub-group were found in the blood of 3 of the 7 mice on the 26th day (the infection in 1 of these 3 mice was also detected by wet preparation on the 56th day). On the 33rd day an additional mouse was found to be infected. All 4 mice remained infected till the end of the study period.

3. Lion 105; adult male: One of the 10 mice showed a *T. brucei* sub-group infection on the 6th day, the strain was frozen (E.A.T.R.O. 1822). No trypanosomes were seen in the remaining 9 mice by wet preparations or thick smears during the 75 days when they were examined.

Five of the 9 mice which were examined on the 26th day by the H.C.T. were found to be infected with trypanosomes (1–4 trypanosomes per 0.06 ml. of tail blood). The same 5 mice were still infected at the end of 75 days and the number of trypanosomes per tube of blood had not changed appreciably.

4. Lion 106; adult female: Five of 8 mice inoculated with blood from this lion showed a very transient low grade infection (1–2 trypanosomes per wet

*Table 1.* Blood examination results for mice inoculated with blood from lion 104, adult male

| Mouse number | Days after inoculation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Wet preparations | | | | | | | Haematocrit |
| | 5 | 6 | 7 | 8 | 9 | 10 | 20 | 26 |
| 709 | 0 | 0 | 0 | 0 | + | | | |
| 710 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 711 B | 0 | 0 | 0 | 0 | ++ | ++ | + | ++ |
| 712 B | 0 | (+) | + | +++ | stabilate E.A.T.R.O. 1811 | | | |
| 713 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (+) |
| 714 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (+) |
| 715 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 716 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 717 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 718 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | +(+) |

B = *T. brucei* subgroup infection.

*Table 2.* Blood examination results for mice inoculated with blood from lion 106, adult female

| Mouse number | Days after inoculation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wet preparation | | | | | | | | | | H.C.T. | |
| | 6 | 7 | 8 | 9 | 10 | 12 | 14 | 15 | 20 | 24 | 24 | 33–75 |
| 729 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (+) | 0 |
| 730 | + | 0 | (+) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (+) | (+) |
| 731 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (+) | (+) |
| 732 | 0 | (+) | (+) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (+) | (+) |
| 733 | 0 | (+) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (+) |
| 734 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (+) | (+) |
| 735 | 0 | (+) | (+) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (+) | (+) |
| 736 | (+) | (+) | (+) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | (+) |

? = haematocrit tube broken.

preparation) from the 6th to the 8th day after which all 8 mice were "negative" by wet preparations and thick smears for the remainder of the study period.

The blood of the 8 mice was also examined by the H.C.T. on the 24th day. Six of the 8 mice were found to be infected with trypanosomes (1–3 trypanosomes per 0.06 ml. of blood). Subsequent examinations indicate that the level of infection had not changed and that 7 mice were infected at the end of 75 days.

## Discussion

In our present study we have encountered 4 low virulent strains of trypanosomes of the *T. congolense* sub-group. At no time during the study (75 days) trypanosomes of this species were seen in wet preparations or thick smears from the mice inoculated with blood from Lion 105 and Hyena 103. These infections would probably have been missed if the H.C.T. had not been used on the blood of the mice. Perhaps higher incidence of *T. congolense* infections might have been recorded in surveys if the H.C.T. had been used to check the blood of the inoculated animals. The obvious advantages of the centrifuge technique are that it is more sensitive, and it saves much time and labour by detecting the infections earlier in the study. By using H.C.T. on the 24/26th day, we were able to demonstrate that Hyena 103 and Lion 104, 105 and 106 were infected with *T. congolense*. If we had relied solely on wet preparations, we would have to wait till the 56th day before we know that Lion 104 was infected with *T. congolense* and we might never have known that Lion 105 and Hyena 103 were infected. Perhaps these infections could have been detected earlier if H.C.T. had been used during the initial stages of the study.

In a preliminary study by one of us (Woo, unpublished data) the blood of a lion (72) and a hyena (71) was examined by this technique in the field at the time the blood was collected for inoculation into mice. A *T. brucei* sub-group infection was demonstrated in the lion but a *T. congolense* sub-group infection in the hyena was missed. This was later confirmed by the inoculated mice. The sensitivity of the technique in detecting low infections could be increased if more than 1 tube of blood (0.06 ml.) was examined from each animal. Further careful study using this technique under field conditions might prove that it can be used directly for the survey of animal trypanosomiasis. In a recent study (Woo 1971) it has been shown that this technique can be adapted for the survey of human trypanosomiasis.

Using the haematocrit centrifuge technique, we have shown that trypanosomes were still present in the blood of mice (inoculated with blood from Lion 103 and 106) that had exhibited a transient infection (as determined by wet preparation). This would indicate that the infection has not been "lost" as is generally believed in such cases. The trypanosomes merely occur in such low numbers that the usual techniques used are not sensitive enough to detect the infection. "Fleeting" *T. vivax* infections in mice or rats are known. The possibility that some of these animals might be infected should be investigated using the centrifuge technique.

Low virulent strains of *T. congolense* such as those encountered in this study, if proven to be also of low virulence in cattle or other domestic animals, might prove to be of value as immunising strains.

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