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Autor: Schütt, I.D. / Mehlitz, D.
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Abteilung für Veterinärmedizin des Bernhard-Nocht-Instituts für Schiffs- und Tropenkrankheiten, Hamburg

On the persistence of human serum resistance and isoenzyme patterns of *Trypanozoon* in experimentally infected pigs[1]

I. D. Schütt, D. Mehlitz

Summary

'Mini-pigs' were infected with salivarian *Trypanozoon* clones to examine the persistence and stability of the human serum resistance [Blood Incubation Infectivity Test (BIIT)] and isoenzyme characteristics during infection in a new host. A stock regarded as *Trypanosoma brucei brucei*, derived from a domestic pig in the Ivory Coast, retained its BIIT negative (serum sensitive), alanine aminotransferase (ALAT) and peptidase 2 (PEP 2) characteristics throughout 343 days of infection in pigs. Similarly there was no change in the BIIT positive (serum resistant) and different ALAT and PEP characteristics of a human isolate from the same area, and regarded as *T. b. gambiense*, during 154 days before the infection became undetectable. In mixed infections of the two clones in pigs, trypanosomes which were not treated with human serum and inoculated into *Mastomys natalensis* invariably displayed the *'T. b. brucei'* characteristics. However, simultaneous inoculations of trypanosomes treated with human serum into *M. natalensis* always displayed the characteristics of the *T. b. gambiense*. Thus, in mixed infections, in which *'T. b. brucei'* predominated, the minority *'T. b. gambiense'* population was recoverable after treatment with human serum by subinoculation into *Mastomys*.

Key words: Trypanozoon; animal reservoir; pigs; gambian Sleeping Sickness; human serum resistance; isoenzymes.

Introduction

Until a decade ago an infraspecific characterization of salivarian trypanosomes of the subgenus *Trypanozoon* was very limited. As these are morphologically identical, they were mainly differentiated by extrinsic features (Lumsden,

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Correspondence: Dr. Ilse-Dore Schütt, Bernhard-Nocht-Institut für Schiffs- und Tropenkrankheiten, Bernhard-Nocht-Strasse 74, D-2000 Hamburg 4

1974) such as geographic distribution, clinical manifestations in man *(Trypanosoma brucei rhodesiense* and *T. b. gambiense)* and infectivity for animals but not for man *(T. b. brucei)* (Hoare, 1972).

Considerable progress – behaviourally and biochemically – was made towards a differentiation of *Trypanozoon* of human and animal origin by testing their resistance to normal human serum (Rickman and Robson, 1970; Mehlitz, 1978) and by comparing their electrophoretic isoenzyme patterns (Godfrey and Kilgour, 1976).

Recently, firm evidence has been presented which indicates gambian Sleeping Sickness is a zoonosis (Mehlitz, 1977, 1978; Gibson et al., 1978). Evidence came from comparing the human serum sensitivity and isoenzyme patterns of *Trypanozoon* stocks isolated from domestic animals and man in West Africa. Human serum-resistant *Trypanozoon* stocks from Liberian pigs proved to have slow alanine aminotransferase (ALAT) isoenzymes which were only known to occur in *T. b. gambiense.*

However, during further epidemiological studies in the Ivory Coast on the role of an animal reservoir of human trypanosomiasis, the presence of two major *Trypanozoon* zymodemes were demonstrated in man as well as in domestic pigs (Mehlitz et al., in preparation): *Trypanozoon* stocks from both man and pigs showed two types of ALAT patterns, type I and corresponding to serum resistant *'T. b. gambiense'* and type II corresponding previously to serum sensitive *'T. b. brucei'* (Godfrey and Kilgour, 1976).

Although it is believed that isoenzymes have a genetically controlled consistent character (Godfrey, 1979), it cannot be excluded that their association with serum resistance and sensitivity may change, or indeed the isoenzyme patterns may change over time as part of the parasite's need for survival, perhaps in a new kind of host.

To investigate this possibility, the distinguishing characteristics of *Trypanozoon* stocks – the resistance to normal human serum and the isoenzyme patterns – were examined as to their stability and persistence in a new relevant experimental host. 'Göttinger Mini Pigs' were infected with *Trypanozoon* clones originating from man and domestic pig in the Ivory Coast. Trypanosomes were re-isolated over a period of one year, and their behavioural and biochemical characteristics were compared to those of the original cloned material used for the experimental infection.

Materials and methods

Experimental animals. 12 'Göttinger Mini Pigs', a special breed for laboratory research purposes, were used for the experiment. All animals were castrated males, 3½ months old. They were fed twice a day with 0.7 kg 'Alleinfutter für Mastschweine' (Ibeka, Hamburg). Water was available ad libitum. The pigs were kept in groups of three in a fly-proofed stable under standardized climatic conditions.

Trypanosomes. Stocks originated from a domestic pig (TSW 116 78E 026) and a human being

(DAL 072), respectively, from the villages Koudougou-Carrefour and Babo (Vavoua-area) in the Ivory Coast. The description of the area is given by Mehlitz et al. (1981). The history of the material for infection is as follows: TSW 116 78E 026 was isolated by inoculation of pig blood into *Mastomys natalensis*. The stock was cloned after the 4th passage in irradiated *M. natalensis* (prepatent period 8 days, then 3 more subpassages, all at 2–3 days after infection during the first increasing parasitaemia). From the last passage the material for infection and characterization was obtained. DAL 072 was isolated by Dr. L. Haller in 1979 by inoculation of human blood into *M. natalensis*. After the 8th passage the stock was cloned and grown in irradiated *M. natalensis* (prepatent period 6 days, then 3 more subpassages, all done during the first increasing parasitaemia), before the material for the experimental infection of the pigs was harvested.

Blood Incubation Infectivity Test (BIIT). The BIIT is based on the trypanocidal action of normal human serum to *T. b. brucei*. Human serum resistant *Trypanozoon* individuals or stocks retain their infectivity to rodents after treatment with the human serum (Rickman and Robson, 1970). Trypanosome populations were tested using *M. natalensis* (Mehlitz, 1978). 3–5 test animals received 0.2 ml parasitized pig blood treated with 0.3 ml human serum each, which was tested for its efficiency with known human resistant (Bida 3) and known human sensitive (LRU TSW 20) stocks. For control animals (3–5) the blood was mixed with 0.3 ml PSG instead of serum. The samples were incubated at 37° C for one hour. The number of inoculated trypanosomes in the different BIIT's varied during the experiment due to changes of parasitaemia in the experimental pigs.

Electrophoresis. For the isoenzyme electrophoresis, trypanosomes were separated from blood by DEAE-cellulose columns (Lanham and Godfrey, 1970) and lysed for thin-layer starch gel electrophoresis as described by Godfrey and Kilgour (1976). The isoenzyme patterns of alanine aminotransferase (ALAT, E.C.2.6.1.2) and peptidase 2 (PEP 2, E.C.3.4.11) were determined throughout the experiment. These enzymes were chosen as indicators for *T. B. gambiense* as their patterns were easy to reproduce and interpret and because of their high enzyme activity even in small lysate quantities.

Parasitology. Pig blood was examined using the haemotocrit centrifugation technique (HCT) (Woo, 1969, modified by Mehlitz, 1978) and wet smears, daily during the first 4 weeks of infection, then twice a week until day 92 after infection. Thereafter, examinations were done weekly up to the end of the observation period (364 days after infection). In the chronic state of infection (from the day 98 after infection onwards) the miniature anion-exchange centrifugation technique (mini-AEC) (Lumsden et al., 1979) was employed additionally. When all methods were negative *M. natalensis* were inoculated with pig blood.

Serum. Blood samples (2.5 ml) were taken from the *Vena cava cranialis* before and after infection once a week for studying the humoral antibody response which will be described separately.

Experimental design

Pigs were divided into 4 groups of 3 animals each (groups I–IV) (Fig. 1). Group I was infected with the *'T. b. brucei'* (TSW 116 78E 026) clone, dosage 1.6×10^5, group II with *'T. b. gambiense'* (DAL 072) clone, dosage 6.1×10^4 trypanosomes/pig. Group III received a mixed infection with the material used for I and II. Group IV served as a control.

The cloned material for infection was tested for human serum resistance and isoenzyme patterns. After infection the BIIT's were performed as often as possible when the pig blood was parasitologically positive. From positive control or test animals of the BIIT's trypanosomes were grown in further subpassuges for preparing lysates. On day 212, 215 and 217 after infection pigs of all groups were immunosuppressed with cyclophosphamide (Endoxan, Asta-Werke, Bielefeld) (12 mg/kg body weight) to try to increase parasitaemia. As the immunosuppression resulted in septicaemia *(Pseudomonas aeruginosa)*, the pigs had to be treated with antibiotics (polymyxin B and gentamycin). Additionally they received symptomatic treatment (methionin, ferrum, coal-tablets).

Results

Course of infection

All pigs of groups I–III developed a parasitaemia after a prepatent perio of 4–5 days. Highest parasite levels were seen in the pigs of group III with mixe infections, the lowest in the pigs infected with DAL 072 (group II), in whic trypanosomes could be demonstrated up to 154 days after infection. Thereafte even the mini-AEC and the subinoculation of blood into *M. natalensis* wer negative. The pigs of the other groups remained positive until at least 364 day after infection (end of the observation period).

In group I, one pig died with septicaemia, following the treatment wit cyclophosphamide; histopathological findings were trypanosome specifi meningoencephalitis and myocarditis. In group II, one pig died 14 days afte intercurrent infection, another one died from a septicaemia after cyclophospha- mide treatment, but no trypanosome specific histopathological changes were found. In group III, two pigs died on days 169 and 212, respectively, due to trypanosomiasis (parasitaemia up to 70 trypanosomes/field, central nervous symptoms, specific histopathological alterations such as meningoencephalitis and myocarditis).

BIIT and electrophoresis

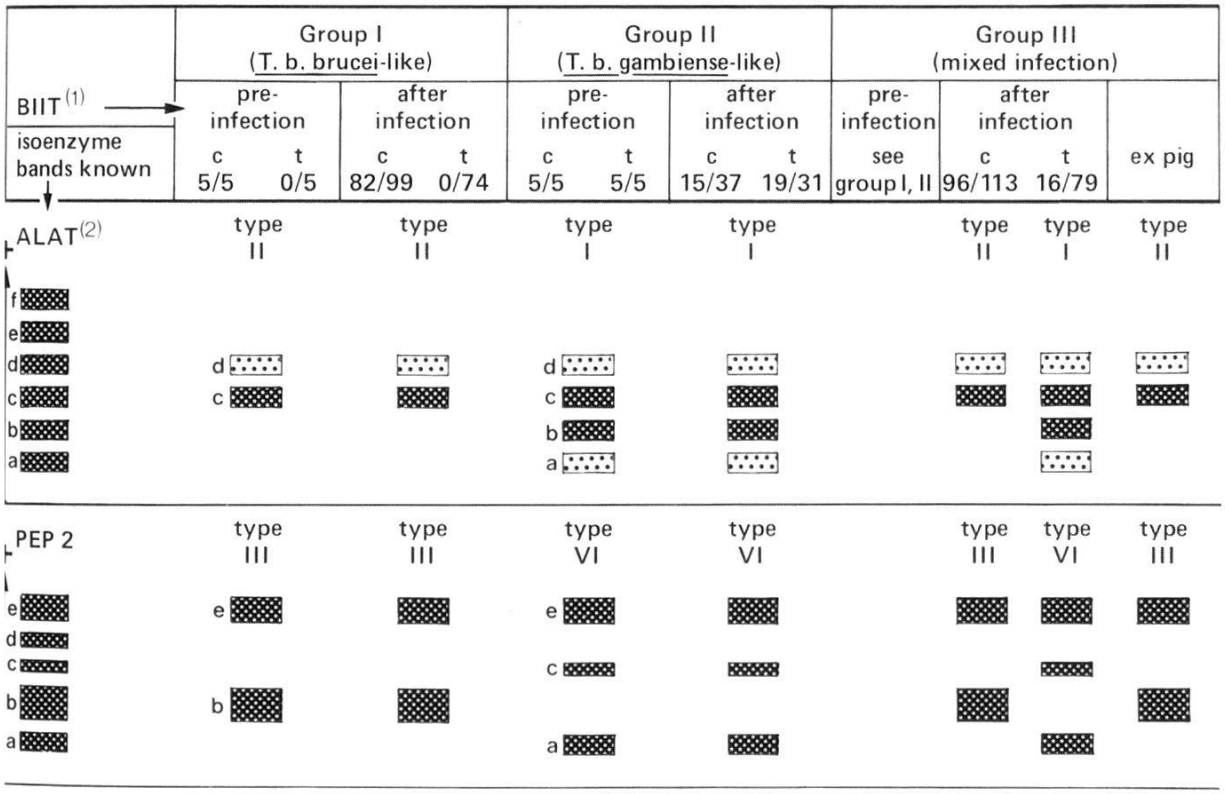
The number of post-infectious BIIT's and thus the number of lysates depended on the parasitaemia in the pigs. Even when some BIIT's were per- formed, the parasitaemia of the control or test animals was not always high enough for preparing lysates. Especially towards the end of the observation period the infectivity of trypanosomes for *M. natalensis* decreased. Summarized BIIT and electrophoresis results are shown in Fig. 1.

Group I *('T. b. brucei')*: A total of 24 BIIT's were done, the first on day 4, the last on day 232 after infection, all with negative results. 82 of 99 control and none of the test animals became parasitologically positive. The isoenzyme pat- terns in 21 lysates were always ALAT type II and PEP 2 type III.

Group II *('T. b. gambiense')*: Only 7 BIIT's could be performed because of low parasitaemias in the pigs, the first on day 4, the last on day 154 after infec- tion. 6 BIIT's had a positive result. 15 of 37 control and 19 of 31 test animals became positive. The fact that only 40.5% of the controls developed parasitae- mia shows the relatively low infectivity of the human derived *Trypanozoon* to rodents. No alterations of the isoenzyme patterns (ALAT type I and PEP 2 type VI) from the lysates of control or test animals could be observed.

Group III (mixed infection): 27 BIIT's were done until day 343; 7 of these became positive, the last one on day 140 after infection. 96 of 113 control and 16 of 79 test animals developed parasitaemia. Lysates prepared from trypanosomes of control animals always produced ALAT type II and PEP 2 type III patterns; lysates from the test animals always produced ALAT type I and PEP 2 type VI.

Fig.1: Summarized BIIT and electrophoresis results before and after infection of pigs with Trypanozoon clones



(1) BIIT: c = control, t = test animals; 5/5 = 5 of 5 M. natalensis parasitologically positive

(2) ALAT and PEP 2 types (patterns) correspond to those given by Gibson et al. (1980)

Bands shown dotted ([:::]) were usually faint and varied considerably in intensity

On 6 occasions when parasitaemia in the pigs was high, lysates could be prepared directly from pig blood, and consistently had ALAT type II and PEP 2 type III patterns.

Discussion

This study showed that behavioural and biochemical characteristics of salivarian *Trypanozoon* clones derived from domestic pig and man did not change under the influence of a new experimental host. The tested characters of human serum resistance and isoenzyme patterns after infection of pigs remained constant for at least 343 days for *'T. b. brucei'* and 154 days for *'T. b. gambiense'*. These findings correspond to those of Kilgour et al. (1975, 1977) who could not find any changes in two isoenzyme patterns (ALAT and ASAT) of *T. vivax* in cattle naturally infected for one year. In contrast, Joshua et al. (1978) made the observation that a tsetse fly derived *T. brucei* stock which did not infect human volunteers became human serum resistant after having been cloned and passed through fowls for more than one year. Further, a change in serum resistance of different cloned antigenic variants of the same trypanosome stock was observed by van Meirvenne et al. (1975) and Rickman (1977).

In the pigs with mixed infection both trypanosomes with the preinfection

characteristics of *'T. b. brucei'* and *'T. b. gambiense'* could be distinguished until 140 days after infection by the treatment of isolations in *M. natalensis* with human serum. Thereafter, only trypanosomes with the characteristics of *T. b. brucei* could be detected. The BIIT proved to be a good tool for selecting trypanosomes with the characteristics of *T. b. gambiense*, as lysates from control animals showed always the pattern of *T.b. brucei* ALAT II, PEP 2 III whereas those from the test animals always showed the *T. b. gambiense* patterns ALAT I, PEP 2 VI. Thus even in mixed infections in which *T. b. brucei* predominated, the minority *T. b. gambiense* population was recoverable.

In pigs infected with the human derived clone trypanosomes were detectable until 154 days after infection but in low numbers. From this it may be concluded that after a single infection pigs can harbour trypanosomes with the *T. b. gambiense* characteristics for at least 5 months and are thus efficient reservoirs even in the presence of *T. b. brucei.* The results support earlier experimental work on the domestic pig as a carrier of *T. b. gambiense* (van Hoof, 1947). The pigs did not show any clinical signs or histopathological alterations due to trypanosomiasis and thus can be regarded as symptomless parasite carriers of sleeping sickness as already been suggested by Watson (1962).

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