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R. Eustace Montgomery & Allan Kinghorn

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ON THE NOMENCLATURE OF THE MAMMALIAN TRYPANOSOMES OBSERVED IN NORTH WESTERN RHODESIA

BY

R. EUSTACE MONTGOMERY, M.R.C.V.S.,

AND

ALLAN KINGHORN, M.B. TORONTO

JOHNSON COLONIAL FELLOW, UNIVERSITY OF LIVERPOOL

Of the Expedition of the Liverpool School of Tropical Medicine to the Zambesi, 1907-1909

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In view of the conclusions at which we arrived as to the nature of the trypanosomes found in bovines of North Western Rhodesia, it appears desirable to briefly outline the subject from the standpoint we have taken.¹ We would observe that at the time our report was despatched from the Luapula we had before us, with small exception, only literature bearing date prior to April, 1907, and on the evidence therein contained, we did not feel justified in discussing the question of the morphology or specificity of our trypanosomes more fully than was done, and it will no doubt have seemed to one noting only the date of publication as though our duty had been somewhat neglected, and our conclusions based in defiance of or without due regard to the results of those workers whose communications had been public property in Europe for upwards of fifteen months prior to the appearance of our report. Had we before us at the present moment only the evidence already advanced, we would hesitate to re-open the question; but the perusal of the literature now with us, and more especially our own more recent observations on trypanosomiasis in other areas of Northern Rhodesia, justify, we consider, this further exposition. In a forthcoming report we shall have occasion to refer to and to compare with that on trypanosomiasis in North Western Rhodesia; and since we purpose utilising this as a basis for critical comparison, it is highly desirable that our position and views

concerning these trypanosomes, to which we have referred as *T. dimorphon* and *T. vivax*, should be most clearly stated ; so that, whatever be the faults in our deductions, these may be recognised and corrected by those to whom the necessary facilities are available. Owing to our protracted absence from Europe and the relative paucity and delay in the receipt of current scientific literature, we shall be precise in specifying all communications referred to, since it is not unlikely that at the time of writing important papers bearing on this particular case may have appeared in print, but will remain inaccessible to us for some months.

Probably no sub-division of the Protozoa presents more difficulties in the way of classification than does the genus *Trypanosoma* Gruby, emended by Laveran and Mesnil in 1901 so as to exclude those organisms of fish which carry both an anterior and a posterior flagellum. On morphological grounds Lühe has created the genus *Trypanozoon* to contain only those trypanosomes parasitic in the blood of Mammals ; beyond this no further attempt has been made to classify or arrange the genus ; and, indeed, any additional sub-division would be impracticable to a Zoologist. We are in a transition stage, when rumours of multiplication cycles, resting stages and forms which from their appearance are unrecognisable, even as flagellates, are abroad. At the present time, we have positive knowledge only of the well-known forms found free-swimming in the blood ; and these presenting such close analogies to one another, offer no opportunity to the Protozoologist of satisfactorily adopting any further classification based on morphological features. It is possible that the more exact and more strictly cytological technique which is now being employed in the study of these organisms may bring to light some new features in their structure to serve as a fixed point from which morphological classification can commence ; but until it has been employed uniformly on all known species, and critical comparisons made, it is of little more value than the older methods used both by Zoologists and Pathologists.

Any classification dependent upon a variable feature and allowing margins to the personal equation must be prone to error, and cannot be unquestionably accepted. Yet we find that Zoologists and Pathologists alike accept as species forms of trypanosomes distinguished from others on morphological grounds, *T. dimorphon*

is admitted to be a 'good species,' and solely from its morphology; *T. equinum* and *T. theileri*, the former more especially, are also constituted largely from their unique appearance in the blood.

A clinician may subdivide the diseases due to trypanosomes on symptomatological or epidemiological grounds: and a pathologist may add to these the results of his observations on animals experimentally infected. Here again absolute distinction between any two species may be impossible, even though it be known that the morphology of each is markedly different. Thus the experimental disease, or the 'animal reactions' due to *T. evansi* and *T. dimorphon*, show very little variation in most Mammals; yet these organisms are clearly distinguishable on morphological grounds. Again, it would be a matter of the utmost difficulty to state specifically that *T. gambiense* was not the same as *T. evansi*, if only dead organisms were available. But if experimental animal reactions are considered, and the natural disease due to each be observed, they can be most strikingly separated.

However lacking in conformity to Zoological rules, and however imperfect and crude, it is possible, by adopting a combination of the results given us by morphological and pathological studies, to constitute various groups of mammalian trypanosomes, which to a worker removed from such facilities as exist in the research centres of Europe are, to our mind, helpful. An observer in the field may place a detected trypanosome, tentatively, in one of the classes with a minimum amount of work, and since he will be in possession of only a modicum of research equipment and a limited stock of experimental animals, he will be in a position to utilise these to their best advantage, and by adopting a certain degree of uniformity can bring his observations into line with all work previously carried out on the class of organisms to which his own approximates. Absolute differentiation of closely related trypanosomes is quite impossible to such a worker: a rigorous and critical morphological comparison must be effected, and final diagnosis will rest upon the result of this and that of 'cross-inoculation' into animals believed to be immunised against the organisms with which comparison is being made.

For such work 'types' of known and approved origin must be at hand, as well as the necessary animals for cross-inoculation. This must remain the work of an acknowledged centre, assuming the

functions of a museum wherein all type species are maintained and strictly guarded.

If we eliminate *T. lewisi* and allied species as not occurring in the blood of the higher mammals, there remain thirteen named species all more or less accepted and distinctive, which an observer may meet in man or the domestic animals. These are *T. gambiense*, *T. evansi*, *T. brucei*, *T. equiperdum*, *T. equinum*, *T. dimorphon*, *T. theileri* (including some of Lingard's forms met in India), *T. vivax*, *T. nanum*, *T. congolense*, *T. cazalboui*, *T. pecaui*, and *T. sudanense*. On morphological grounds it is possible to distinguish :—

T. theileri, on account of its relatively immense size, and further from its animal reactions, being parasitic only in the blood of bovines. Dutton and Todd, however, describe a trypanosome morphologically recalling this organism as occurring in the blood of a *Tragelaphus scriptus* in the Congo Free State.²

T. equinum, which in stained preparation (dry method) shows a uniquely small blepharoplast. On epidemiological grounds in addition, by being limited to South America.

By adopting certain standards of animal reaction, we can regard as distinctive :—

T. gambiense, as being the only one, so far as known, pathogenic to man. The human subject not being available for inoculation, the diagnosis of forms recalling this species morphologically would remain work for comparison with types.

T. equiperdum, on the grounds of production in equines of quite characteristic clinical symptoms ; and being unique among trypanosomiasis of the lower animals in transference naturally by coitus.

Nine named species remain to be dealt with. By the adoption of morphological and animal reaction standards these can be subdivided into three groups, having as their types, in point of view of priority, *T. evansi*, *T. dimorphon*, and *T. nanum*, respectively.

The standards we adopt are arbitrary, and are open to criticism, as any must be which depend upon more or less relative features of size and pathogenicity.

Considerable variations are noticeable in the appearance of individual trypanosomes of any strain: we have the so-called 'male' and 'female' elements; and gross measurements are found to vary. But, be it noted, these variations are within limits. It would be most exceptional to find, for instance, any *T. evansi* of less than 20μ or of more than 35μ in length; and despite the tens of generations which many trypanosome strains have produced in various laboratories, we find the morphological features remain practically constant. There is not that tendency to develop new types, to revert to an old form, or otherwise to vary, which has more than once been suggested. We may with comparative safety, then, group into one class some of those nine species which conform approximately to the dimensions of *T. evansi*. Disregarding the differences in 'male', 'female' and 'indifferent' forms, these species would be monomorphic and of relatively large size.

On the other hand, there is a group of three species the morphological appearances of which differ markedly. In the course of infection by *T. dimorphon*, forms closely resembling *T. evansi* are encountered, but at the same time, or in the same animal, or, capable of production in another animal, trypanosomes markedly smaller, measuring only 10μ to 15μ in length, and without any free flagellum, make their appearance. It has been shown that these are but various manifestations of the same organism, which is consequently far from being monomorphic.

Of the nine species to which we refer, six show similar reactions towards experimental laboratory animals; three differ markedly. The value of animal reactions may be disputed, and when limited in amount, results are apt to cause confusion. Particularly is this the case with the donkey, cattle, sheep and goats, largely, we think, owing to the multitude of races and breeds employed, and also because many of them are country-bred animals whose ancestors have probably been exposed for generations to trypanosome infections. These animals, too, show a degree of individual idiosyncrasy which is prone to give rise to error. Monkeys, especially *Cercopithecus* and *rhesus*, domestic rabbits, guinea-pigs, white rats and tame mice, and to a slightly less degree, dogs of both European and native blood, are, however, fairly constant in their susceptibility or their insusceptibility to any one strain of trypanosome. During the many generations

which have been maintained of such organisms as *T. gambiense*, *T. evansi* and *T. dimorphon* in all the usual laboratory animals, there has never been any suggestion that a new type has been created. Increased and decreased virulence may and does occur, but this is less marked, under normal maintenance of the strain, than was hitherto supposed, and there is always a tendency to reversion towards the original state.

By means of these two factors the nine remaining trypanosomes can be conveniently grouped as follows:—

- A. Trypanosomes, pathogenic towards most domestic animals, and producing a rapidly fatal infection in the usual laboratory animals :
 - (a) monomorphic, of large and fairly constant size, from 20μ to 35μ in length, and carrying a distinct free flagellum. Type—*T. evansi*.
 - (b) di- or polymorphic, of very variable size, occurring in at least two forms: (1) 'short,' measuring from 10μ to 15μ in length, and devoid of a free flagellum; (2) 'long,' which may attain 35μ , and bearing a variable length of flagellum. Intermediate forms also occur. Type—*T. dimorphon*.
- B. Trypanosomes, pathogenic to certain domestic animals, and without apparent effect when inoculated into the usual laboratory animals (monkey, dog, rabbit, guinea-pig, rat and mouse). An imperfectly studied group, which includes *T. nanum*, *T. vivax* and *T. cazalboui*.

We consider this grouping to be sufficiently exact for the temporary purposes of a worker in the Tropics. Unless the organism with which he is dealing shows any striking peculiarity in either morphology or animal reaction, he can do little more than assign it to one of these groups.

In North Western Rhodesia we isolated from cattle, sheep and dogs three trypanosomes, one approximating to each of the groups, and we referred to these as *T. brucei*, *T. dimorphon* and *T. vivax* for the reasons set out below.

- i. *T. evansi* group, which includes *T. brucei* and *T. sudanense*.³

The validity of *T. brucei*, save on the grounds of cross-inoculation, is open to question, unless it be pre-surmised that each species of trypanosome has its own particular genus or species of biting fly to bring about dissemination. In Africa, where both *T. brucei* and *T. evansi* occur, it has become usual to refer to *Glossina* and *Tabanidae* as the respective transmitters. In the same manner an *evansi*-like organism, which in nature is supposedly spread by *Glossina*, or which is met with in cases of 'tsetse-fly disease,' is referred to as *Trypanosoma brucei*. This, we take it, implies the acceptance of *T. brucei* as the local type of the *evansi* group, and we consequently assigned to it our dog trypanosome. But it is no more possible to distinguish between *T. evansi* and *T. brucei* in the field, unless the supposed transmitting factor be considered, than it is to differentiate *T. evansi* and *T. sudanense*.

2. *T. dimorphon* group, including *T. congolense*⁴ and *T. pecaui*.³

In their action on laboratory animals these three species coincide very closely; and in their morphology, as shown in stained film (dry method), *T. congolense* is almost identical with the *T. dimorphon* at Paris. Writing on the subject of these two, Martin, Leboeuf and Roubaud⁵ hold that in the French Congo the latter is more active, moving more readily across the field and producing more extensive lateral displacement of the corpuscles; and they contend that a little experience will enable an observer to distinguish between the two. Assuming that these two species are distinct, based on the strains in Europe, it is not to be forgotten that in countries such as parts of Africa, where mixed infections are not unknown, they might conceivably both occur in the same host, and here more especially since the geographical distributions coincide.

T. pecaui appears to have more claim to recognition in the field. Morphologically, the similarity between it and *T. dimorphon* is great, although it has been noted that the 'short' form of *T. pecaui* may attain a greater breadth and carry a slightly better developed undulating membrane than is usual in the type strain, and both forms are present at the same time. Laveran,³ however, states, 'these morphological differences do not suffice to differentiate the two

parasites.' It will be remembered that Dutton and Todd⁶ were unable to adduce much information regarding the animal reactions in the course of the natural disease of the Gambia, except in horses. These correspond to what are manifested in 'baleri' as outlined by Cazalbou.⁷ In experimental animals the results coincide: the disease is acutely fatal in rats, guinea-pigs and dogs, though Laveran has noticed some slight variations in the mouse, and in all animals, as in *dimorphon* and *congolense* infections, splenic enlargement is common. One further point of difference might be cited; in sheep and goats experimentally infected with *T. pecaudi*, the blood, though virulent on subinoculation, very rarely shows parasites; in *T. dimorphon* infection this is not usual. We have already referred to the variability of these animals under experimentation.

The parasite which was obtained in the majority of cattle at Broken Hill corresponded to the original description given by Dutton and Todd of *T. dimorphon*, in so far that all three forms, including that with a free flagellum, were found, and the animal reactions were similar. Since the free flagellated and the small forms were not present simultaneously, it is improbable that we were dealing with *T. pecaudi*; and as *T. congolense* does not appear to occur in a 'long' form it may also be negated.

3. Group including *T. nanum*, *T. vivax* and *T. cazalboui*.

This group, constituted on the grounds of immunity enjoyed by the usual laboratory animals, presents difficulties in the way of subdivision, as from the very nature of the parasites they are more difficult of use in experimental observation, and hence less studied.

Of the three species, *T. nanum* takes priority; but, if we may judge from the very limited amount of work that has been possible, it is clearly separable from the other two on account of its morphological features. Laveran⁸ describes it as only measuring 10 μ to 14 μ in length, and this in conjunction with Balfour's observations⁹ that two monkeys, two rabbits and a dog were not infected are held to substantiate the species. It is true that the morphological appearances strikingly recall the 'tadpole' form of *T. dimorphon*; but though the number of inoculated animals be small, infection, in some at least, should have resulted had this organism, to which laboratory animals are highly susceptible, been employed. Further

work may regain *T. nanum* in the Sudan, when its position may be made more clear; until that time its specificity must remain questionable, though we incline to consider it as distinct from *T. dimorphon*, and, therefore, to have no relation to the trypanosome of North Western Rhodesia which we have associated with that species.* Further, its small dimensions preclude the possibility of its connection with either *T. vivax* or *T. cazalboui*.

In July, 1906, Laveran¹⁰ announced his belief that the trypanosome of 'La Soumaya' was a new species, to which he gave the name *T. cazalboui*. Up to this time the impression had been created by various writers that the causal agent of La Soumaya was *T. evansi*, and, indeed, such statements had been made. Laveran and Mesnil¹¹ say 'ils ont la plus grande ressemblance avec le trypanosome de la Mbori,' and they proceed to quote Cazalbou's animal inoculations which resulted in the death of grey rats, mice and Sudanese dogs. In a later paper, published in May, 1907, Laveran³ advances such additional evidence as to make the species incontestible. By morphology, animal reactions and cross-inoculations, *T. cazalboui* is clearly separated from the *evansi* and *dimorphon* groups, and from *T. nanum*, to which it is only related by the similarity of animal reactions. On all grounds it is a 'good species,' and one which can be detected and classified in the field with comparatively little trouble.

The same can hardly be said of *T. vivax*, as we yet know it, an organism described by Ziemann in 1905, a year before *T. cazalboui* was created, and given a specific name mainly on account of its rapidity of motion in cover-glass preparations. The morphology of this organism is in dispute: Ziemann¹² distinguishes it from *T. brucei*, though Laveran¹³ was unable to note any difference from *T. evansi* in the film he examined; and Schilling¹⁴ contends that it is but a slightly more rapid form of *T. brucei*. This opinion is not reflected by Lühe, who writes¹⁵ 'Ich selbst finde in einem mir übersandten 'Originalpräparate die unter sich durchaus gleich gestalteten 'Trypanosomen kleiner wie *Tryp. brucei* . . . mit nur schwach 'ausgebildeter undulierender Membran, wenig hervortretenden

* Wenyon (Report of the Wellcome Research Labs., Vol. 3) has apparently recovered *T. nanum*. We consider that one of the trypanosomes we have isolated from cattle, sheep and goats in North Eastern Rhodesia is closely allied to this species. (April 15th, 1909.)

'Bandsaum derselben sehr kurzer oder völlig fehlender freier Geißel, 'rundem Blepharoblast und nicht auffällig zugespitztem Hinterende.' 'Die Länge beträgt nach Ziemann 18-26, bisweilen bis zu 30 μ , die 'Breite 2-2 $\frac{1}{2}\mu$; der runde Blepharoblast liegt nahe dem meist etwas 'zugespitzten Hinterende.' To this we may add that Ziemann found difficulty in differentiating 'sexual' forms; an implication that this trypanosome is not subject to morphological variations of more than slight degree. Writing on *Trypanosoma cazalboui*, Laveran says 'its length, including flagellum, is 21 μ , breadth 1.5 μ . 'Nucleus oval and situated towards the middle. The centrosome 'round and distinct is placed near the posterior extremity, which is 'rounded and not pointed. Undulating membrane is very slightly 'developed, being little folded as in *T. lewisi*.'

A comparison of Lühe's and Laveran's descriptions of *T. vivax* and *T. cazalboui*, respectively, shows how little morphological difference there is between the organisms in stained film; and the similarity is accentuated when, referring to the movement in fresh preparation, Ziemann's organism, named on account of its motility, is described as moving "'wie einen Hecht" in mehr oder weniger 'gerader Linie quer durch das Gesichtsfeld schießen lässt;,' while *T. cazalboui* is said to be 'very active, moving sometimes on itself, 'at others soon leaving the field like an arrow.'

The animal reactions of *T. vivax* have been imperfectly studied. Comparing the natural disease induced in cattle, sheep and goats, we can note no great difference from La Soumaya. Experimentally, Ziemann is quoted by Sander and Hennig¹⁶ as having had the following results: 'Graue Ratten, Tod nach 8-11 Tagen; Deutscher 'Hund (?); einheimischer Schweine: nur leichte Erkrankung; 'Esel: chronischer Verlauf . . . Ohne Erfolg; Katzen, Haus- 'geflügel, eine weisse Ratte.' Nabarro in his analysis of Ziemann's paper writes¹⁷: 'Dogs, cats and pigs were found not to suffer from 'the natural infection.' 'A dog and a native sucking-pig developed 'a slight temporary infection. White rats, geese, ducks, native hens, 'young turkeys, a native cat and an old pig were all refractory.' Despite the limitations of Ziemann's opportunities, it remains unquestionable that he was dealing with an organism showing marked lack of pathogenicity towards experimental animals. Laveran has not recorded the results following inoculation in grey rats, but

Cazalbou had previously noted their susceptibility to the trypanosome of La Soumaya.

It is most certainly to be regretted that *T. vivax* has not been placed on a more substantial footing; but the evidence that is before us indicates strongly that it has few if any affinities with either the *evansi* or *dimorphon* group, and we are unable to see wherein *T. cazalboui* differs in any manner from what has been made known regarding *T. vivax*, and as further indicating the resemblance between these two, it may be added that Tabanidae are blamed by the German writer for transmitting his species in the Cameroons, and Cazalbou on more than one occasion lately has emphasised his belief that this family is concerned in the dissemination of La Soumaya in the Niger regions.

Though they have never received specific designations, two other trypanosomiasis of cattle deserve mention in connection with this group: that of Bruce, Nabarro and Greig at Entebbe,¹⁸ and that described from Erythrea by Memmo, Martoglio and Adani.¹⁹ In each instance the respective observers have noted the insusceptibility of laboratory animals to infection. The following paragraph sums up the morphology of the Erythrean organism:—'The trypanosome 'is morphologically like *T. brucei* or *T. evansi*, but is not more than '24 μ long; free flagellum, which is fairly long, included. It is 'extremely motile, like *T. vivax* of Ziemann. This trypanosomiasis 'appears to be very virulent for ruminants, and thus differs from 'typical surra. In many respects it resembles the disease described 'by Cazalbou in French Sudan under the name Souma. There is no 'tsetse in the infected area, and the suspected fly is a *Tabanus* or a '*Hippobosca*.'

It appears to us that these two species are incapable of absolute diagnosis, save, perhaps, after a rigorous critical comparison. This to us in Africa is impossible, and we have consequently associated our second cattle trypanosome with the senior member. We would add that Broden⁴ has also been struck by the unusual similarity between *T. cazalboui* and *T. vivax*, which he considers he has obtained in the Congo.

KAMBOLE, NORTH EASTERN RHODESIA,

October 5th, 1908.

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