A Possible Vector of *Endotrypanum schaudinni* of the Sloth *Choloepus hoffmanni*, in Panama

Endotrypanum schaudinni, a haemoflagellate of sloths, has a very wide geographical distribution in America, stretching from the Amazonian Basin in the South to Costa Rica in the North. It has been shown that the infection is limited to animals which live in forest regions[1]. Sloths of the genera *Bradypus* (three-toed sloths) and *Choloepus* (two-toed sloths) from the Atlantic side of the Isthmus of Panama were examined, and 54 per cent of the two-toed sloths were found infected with *E. schaudinni*. This area was, therefore, highly endemic for endotrypaniasis, suggesting that a highly efficient transmission was occurring at the time. No infection was found, however, in 11 three-toed sloths, but this genus apparently seldom enters the endemic rain-forest habitat.

Using a two-toed sloth as 'bait', an attempt was made to find the vector of *Endotrypanum*. The sloth was placed on a platform 30 ft. up a large tree in the rain-forest, and any insects found feeding on the animal were collected and taken to the laboratory for dissection and identification. A wide range of insects was found, the commonest being species of mosquito, horsefly and sandfly. The greatest number of flies of any one genus found, over a period of 24 h. were *Phlebotomus*. Large numbers of these flies were collected in short periods of time, but even minimal change of optimum feeding conditions (for example, the slightest breath of wind) would completely disperse them.

In some of the *Phlebotomus*, dissected the day after feeding, active peripheral blood forms of *E. schaudinni* were seen in the blood meal. These represented a 'fortuitous xenodiagnosis', for at the time it was not known that the sloth was infected. *Endotrypanum* was later identified by stained blood films, in fresh blood and in cultures from the animal.

Typical blood forms of *E. schaudinni*, now extracellular, were still present in the stomach of *Phlebotomus* three days after the insects had fed. In addition there

were a few extracellular rounded forms—morphologically similar to the initial stages seen in primary isolations in blood agar cultures obtained from infected sloths. Leptomonads were found in the remnants of the blood meal of a *P. sanguinarius* which had fed on the infected sloth 4 days earlier. The blood meal and flagellates were still enclosed in the peritrophic membrane, while the rest of the intestine was completely free of flagellates. It may be presumed, therefore, that the parasites were derived from the blood meal.

Leptomonads were also demonstrated in 21 per cent of the *Phlebotomus* species taken from human 'bait' on the same platform. These infections were typified by small rosettes throughout the hind gut. More active single leptomonads were seen in the lumen of the hind gut and, in some flies, in the lumen of the Malpighian tubules. It could be argued that this infection in the wild flies was an insect *Leptomonas* species, but this is considered unlikely, as of 262 male *Phlebotomus* dissected in the past few years, none had flagellate infections[5].

Further evidence of the development of *E. schaudinni* in *Phlebotomus* was obtained by feeding laboratory-reared *P. sanguinarius* on the sloth. On the third day, the blood meal still contained *Endotrypanum* peripheral blood forms. In one fly, on the sixth day, the infection was found in the hind gut and consisted of twelve rosettes of flagellates. The rosettes were small and composed of no more than 4 organisms in each group. The station and nature of the flagellates presented, therefore, an identical picture to some of the infections observed in wild-caught flies.

In the other insects found feeding on the sloth, there was no multiplication of the parasite. In some clean laboratory triatomid bugs, experimentally fed on the same and other sloths, the active forms did not survive for more than 24 h. These results confirm in full the findings of Cunha and Muniz[3], Montero-Gei[4] and Deane[5].

It could further be argued that the sloth may have had an unsuspected *Leishmania* infection, and, if this were the case, it would follow that the infection in the sandflies was a *Leishmania* and not *Endotrypanum*. For the reasons which follow, it would seem to us that we were dealing with stages of *Endotrypanum* and not a *Leishmania*.

The sloth has never been reported to be a host of *Leishmania*. Cultures made from sloths with *Endotrypanum* in their peripheral blood invariably produced leptomonads identical to those discussed. From other sloths, in which peripheral blood forms of *Endotrypanum* were absent, consistently negative cultures were obtained. These observations are based on the examination of 150 sloths from many localities. The strains of *E. schaudinni* isolated from the 'bait sloth' and other sloths have, as yet, failed to infect hamsters and other laboratory animals. This further supports the view that the leptomonad isolations were not members of the genus *Leishmania*. Further, no typical tissue stages of *Leishmania* were demonstrated in impression smears of the internal organs from sloths infected with *E. schaudinni*.

The absence of anterior infections in both the clean experimentally infected flies and the wild naturally infected flies is also atypical of the genus *Leishmania*[6]. Finally, it has been shown that endotrypaniasis is endemic in forest regions[5]. In this habitat the most common genus of insects found feeding on sloths was *Phlebotomus*. The species involved in this study (*P. gomezi*, *P. sanguinarius*, *P. trapidoi* and *P. ylephiletor*) all feed arboreally and are rain-forest species, as are most of the species recorded from this part of the world[7]. In open regions of the same locality, sandflies were not found feeding on sloths or human beings. Where the possibility of contact with the tropical forest habitat was unlikely, or their habits precluded them from the endemic regions, sloths were uniformly negative for infections of *E. schaudinni*.

It is often difficult to identify with any certainty flagellates found in the alimentary tract of wild-caught blood-sucking insects. Such infections may be either a species of flagellate that is restricted to the insect, or a stage in the life-history of a flagellate that is also parasitic in vertebrates. In a recent publication, McConnell[8] and his colleagues stated that out of 18 leptomonad isolations from wild-caught *Phlebotomus*, only 4 produced Leishman-Donovan bodies when they were inoculated into hamsters. Furthermore, using the agar-diffusion precipitin technique, a complete lack of cross-reaction was noted between a Panamanian human strain of *Leishmania* and two leptomonad isolates from wild-caught *Phlebotomus*, similar to those that had failed to infect hamsters. This work indicates that more than one species of leptomonad flagellate is present in the wild-caught *Phlebotomus* of Panama. That some of the sandfly leptomonad infections reported from this region[2,8] are members of the genus *Leishmania* seems certain, but so far as the nature of the majority of the infections are concerned it is only possible to say that they are perhaps mammalian in origin[8].

The present evidence suggesting *Phlebotomus* as the natural vector of *E. schaudinni* is supported by the work of McConnell[8], and in turn offers an explanation as to the origin of at least some of these wild *Phlebotomus* leptomonad infections that could not be incriminated as stages in the life-cycle of a *Leishmania*.

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