Leeches- Guide to Survey of African Trypanosomiasis In Wild Animals in Niger Delta Nigeria

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Abstract: Transmission of trypanosome is traditionally related to vectors, Glossina, Tarbanids and Stomoxys but is implicated in leeches. Increasing seropositive cases of Human African Trypanosome without corresponding vector implication has been noted in Abraka, Nigeria. This fails to explain the epidemiological relation between vector presence and humans infected with African Trypanosomiasis in the study area. Surveillance of trypanosome among wild animals may be complex and difficult. Besides, wild life such as Bushbuck and Hogs are known to be healthy carriers of trypanosomes and can sustain transmission in both human and livestock. A total of 12 earthen fish ponds in the swampy wild area of Ogono village in Abraka, Nigeria were surveyed for the presence of leech parasites. Over 250 leeches were harvested with an average of 20 leeches from each pond. In the laboratory, examination of gut aspirates of leeches using tuberculin syringe revealed dark sanguineous fluid after being fed using laboratory rats and observed for 32 weeks. This suggests that digested blood from its several preys can be aspirated and analyzed for DNA extraction. Hence DNA analysis of gut content could bring about knowledge of the presence of the wild reservoir hosts in areas of surveillance. Also, DNA analysis to identify the presence of trypanosomes in leeches which may have fed on trypanosome infected bushbuck, hog or other aquatic animals which are known reservoir of African trypanosomiasis, could facilitate the surveillance and the epidemiology of trypanosomiasis in wild animals.

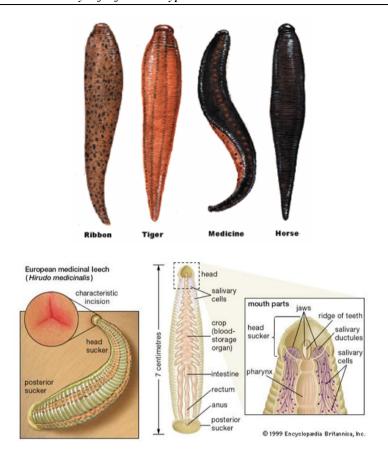
I. Introduction

Abraka is located in Ethiope East Local Government Area of Delta State, Nigeria. It is located in latitude 546'59.988 " N and longitude 65 ' 60.000" E (www.tiptopglobe.com/city?n=Abraka&p=6940). Abraka has a tropical wet and dry climate, with a lengthy wet season which runs from March through October. A lull of precipitation occurs in August.

Wild life such as, Waterbuck, Bushbuck and Hogs, Bovidae, Lions are known to be healthy carriers of trypanosomes and can sustain transmission in both human and livestock (Anderson *et al.*, 2011). The increasing cases of Human African Trypanosomiasis in Abraka (Enwenzor and Ukah, 2001) without corresponding traditional vectors like Glossina, Tarbanids and Stomoxys prompted the search for other possible vectors in this area. Leeches are segmented worms in the Subclass Hirudinea that are usually ectoparasitic (Baskova, 2004). Endoparasitism also exists in leeches (Farhad, *et al.*, 2009). There are 500 to 1000 species of leeches worldwide (Govedich and Bain, 2005). Leech habit in freshwater (slowly flowing and rarely fast running water), moist terrestrial, marine and amphibious species also occur (www.animals.jrank.org/pages/1692/Leeches-Hirudinea-HABITAT.html">Leeches: Hirudinea - Habitat). Leeches cannot live in contaminated and smelly water (Govedich and Bain, 2005). Most Leeches are hematophagous (Sawyer, *et al* 1986) and feed infrequently but consume a large quantity of blood at a time from a wide variety of animal hosts ranging from fish, wild mammals to human. Leech excretes plasma from their ingested blood meals, thereby increasing the proportion of blood cells in the ingesta. Studies have shown that digestion of blood content in leech takes place very slowly and that concentrated blood cells in the gut of leech can survive for 6 months in cases in which leeches were stored at 30*C (Nehili, 1994).

The long time survival of blood cells in leech is an indication that, host DNA can be extracted to track down animals in the wild relevant in the transmission of African Trypanosomes. (Bertelsen and Thomsen, 2012)

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II. Materials and Methods

Twelve (12) earthen fish ponds were investigated for the presence of leeches. The ponds were located in the swampy forest of Ogono village in Abraka, Delta State. With the aid of metallic plate, mud was collected from the base of the pond into a plastic sieve, and leeches were picked into labeled cylindrical glasses which were half filled with water from the ponds.

In the laboratory, with aid of tuberculin syringes, aspirate was taken from the gut of the leeches and examined by wet preparation macroscopically. Leeches were maintained in the laboratory in glass aquaria filled with pond water. Twenty one (21) laboratory rats were shaved in the hind limbs and pelvic region. Each shaved rat was introduced into a cylindrical glass of water containing 4 leeches which were allowed to engorge to satisfaction (self disengagement from the host). Leeches were kept away from feeding for 32 weeks at room temperature. Twice weekly, for 32 weeks body fluids were taken from 2 previously engorged leeches and examined by wet preparation macroscopically.

III. Results and Discussion

Sanguineous fluid was found in all freshly harvested leeches. Following feeding from laboratory rats, whole blood was observed in leeches up till the 6th week post engorgement. Aspirates were gradually replaced by dark body fluid which was observed up to 20th week. Leech concentrates blood cells by removal of water and excessive ion via paired nephredia (Nehili, 1994). The digestion of concentrated cellular component takes place rather very slowly. This confirms again work done by Nehili (1994) that ingested blood cells survive for long periods, in 6 months in cases in which leeches were stored at 3^oC. Same effect was reported by Al-Khleif, (2011) for *Bovine Parvovirus* (BPV), *Feline Calici virus*(FCV), *Equine Arteritis* Virus (EAV) and *Equine Herpes virus*type 1 (EHV-1) pathogens. Also,for African mammalian trypanosomes, *T. brucei and T. congolenses* (*Odoya et al*,2004). *Aeromonashydrophila*(natural flora) and human pathogens (Ahl-Khleif, 2011); HIV and hepatitis B in African leeches from Cameroon (Nehili, *et al.*, 1994).

Protozoa parasites, *Toxoplasma gondii*, *T.brucei brucei and Plasmodium* are known to survive in leech gut (Nehili *et al*, 1994). Observation of the bite marks on the body of the rats showed grasping. This was confirmed by dissection which showed that their mouth parts were arranged by an upper and lower lip and a number of small teeth along its jaw.

Leech consumes the blood of a variety of animals host ranging from fish to human but prefers mammalian blood possibly because of its high energy content (Sawyer, 1986). Leeches could be terrestrial, amphibious and marine. Laboratory rats experimentally infected with trypanosomes and allowed to be fed by leeches confirms that trypananosomes can survive in the gut content of leeches (Odoya *et al* 2004). The confirmation of DNA component of wild animals in the gut content of leeches (Ewen, 2012) gives a probable confirmation of the DNA constituent of trypanosome in the blood of leeches. Thus, DNA analysis to identify the presence of trypanosomes in leeches which may have fed on trypanosome infected bushbuck, hog or other aquatic animals which are known reservoir of African trypanospomiasis, could facilitate the surveillance and the epidemiology of trypanosomiasis in wild animals.

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