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INTRODUCTION

Trypanosomes are inoculated into a host (human or animal) in saliva via the biting mouth parts of the tsetse fly. Trypanosomes circulate in the blood of their host, differentiate and spread to other tissues via the lymphatic system during a 30 day period. Humans with African Sleeping Sickness or Human African Trypanosomiasis (HAT) complain of having a fever, headaches and joint pain. If left untreated, a neurological phase develops causing confusion, fatigue, narcolepsy like symptoms, irreversible nerve damage and eventual death. Often associated with malnutrition and poverty, HAT has been reported to have been in existence in Africa since the 14th century. HAT has wreaked havoc and affected millions of lives since that time. Three devastating epidemic waves have occurred since the 1890s in southeast Uganda alone.[1] Between 1902 and 1903 the disease agent, a protozoa, was determined to be transmitted by the tsetse fly (*Glossinia sp.*) and are morphologically the salivarian clade trypanosomes (i.e. their life cycle involves the salivary glands of the tsetse fly).[4] Three subspecies of *Trypanosoma brucei* sensu lato (s.l.) include *Trypanosoma brucei rhodesiense* (Southern and Eastern Africa) and *Trypanosoma brucei gambiense* (Central and Western Africa), which are infective to humans and *Trypanosoma brucei brucei*, which is not infective to humans, but is to animals. There is uncertainty as to whether *T.b. gambiense* is zoonotic, if it has a wild animal reservoir that will transmit sleeping sickness to humans or whether it is just transmitted between humans. However, cattle have been implicated as the primary reservoir for *T.b. rhodesiense*. It is known that clinical manifestation of disease in cattle is caused by *T. vivax* and *T. congolense*. Cattle become anemic, develop a dull attitude, rough hair coat, succumb to severe debilitation due to emaciation and become weak and die if left untreated.

Although wild animal species are reservoirs for trypanosomes, it is commonly accepted that cattle and swine, which live in close association with man are the main reservoir hosts. Epidemiology papers have been produced regarding HAT or cattle trypanosomiasis with emphasis on the tsetse fly vector, its ability to transmit the protozoan organism to humans and the environment in which it thrives. Few epidemiologic studies have been conducted showing the relationship between the trypanosome-infected cattle reservoirs and the human host. As many people live in close proximity to their cattle, this paper will focus on the epidemiologic relationship between trypanosomes, humans and the animals they keep for their livelihood, survival and income.

LITERATURE REVIEW

Cross-sectional trypanosomiasis studies between humans and animals have been difficult to ascertain due to war, famine and political or economical corruption in tsetse-infested areas of Africa. Naturally, it is unethical to spearhead studies utilizing human subjects in this regard.

The origins of a new *Trypanosoma brucei rhodesiense* sleeping sickness outbreak in eastern Uganda.

Although the tsetse fly *Glossina fuscipes fuscipes* existed in large numbers in the Soroti district of southeast Uganda, it was a relatively sleeping sickness free area albeit one case reported in the mid-1960s. (This case was found to have its origins from the south). Civil war broke out in 1979, which was soon followed by civil unrest. Many people and their animals moved out of the area. Once civil stability was re-established, cattle (unscreened for trypanosome sp.) were imported into Soroti. Fèvre et al (2001) tested the hypothesis that an epidemic outbreak of Human African Trypanosomiasis (HAT) resulted from cattle infected with *T. b. rhodesiense* imported from HAT endemic areas. They also looked at the distance of infected persons to the Brookes Corner market where cattle were sold as a risk factor for HAT on a cluster map.

A retrospective matched case-control study was implemented in June 2000. The first HAT case was reported on 31 December, 1998 and tsetse control measures were established between February and July, 1999. However, 119 cases of HAT were reported between 31 December, 1998 and 2 June, 2000 at the catchment area of Serere Health Centre. Data from patients with complete medical records and a history of never having traveled outside of Soroti District was collected. Each of the 113 out of 119 that fulfilled the above criteria (mean age 29.8 years, SD 19.9 years, 60% male), were matched with one control by age, sex and month. All persons not only lived in the Serere Health Centre catchment area, but were admitted to the Serere Health Centre. Some 2,796 head of cattle were traded between April, 1995 and December, 1998 at Brookes Corner market of which 1,510 originated from *T. b. rhodesiense* endemic areas to the south.

Using cluster detection and statistical analysis the authors determined that the increasing distance from Brookes Corner market was significantly associated with lower risk. When tested individually, elapsed time from the start of the outbreak until actual infection was reported as not being significant. However when distance and time were tested, there was high significance demonstrating that distribution risks did change with time as cases reported moved away from the Brookes Corner market. The results demonstrated that there was an association between human infective parasites and the movement of cattle to a previously naïve HAT area. Cluster maps demonstrated that cases of HAT were in villages close to the Brookes Corner market within the first 30 days of imported cattle arriving prior to vector control. Tsetse blood meals were primarily from cattle, but given the acquisition of cattle and their location in villages within a focal area, the tsetse were able to transmit trypanosomes to human hosts in the villages nearby. This was an excellent paper showing the interaction of reservoirs and hosts – trypanosome infection in cattle and African Sleeping Sickness.

As infections continued in spite of vector control measures in the Soroti District, the authors were able to determine that reservoirs play a major role in the persistent nature of HAT. This paper describes events four years after those described above. Violence and instability occurred again during 2003 in the Soroti District. This disrupted the treatment of livestock with long-acting trypanocides and vector controls causing a perpetuation of the HAT epidemic. The authors screened 335 animals for trypanosome infections (using PCR to detect *T. brucei* s.l. and *T.b. rhodesiense* alone) from three villages in the non-intervention area and from three villages having reported HAT before interventions were started. Fifty-one animals were also sampled from Brookes Corner market on one day. Using binomial regression, confidence intervals were determined resulting in high standard errors in combination with uncorrected X^2 values, leading the authors to conclude that human *T. b. rhodesiense* in the cattle populations where there were interventions were not significantly different from areas where there had been no intervention.

Mass treatment of animals is necessary to prevent parasite transmission to tsetse in addition to vector control to prevent transmission of trypanosomes to humans. Animals at the market were still being imported from endemic areas with *T.b. rhodesiense*. Although not highly pathogenic to local cattle breeds, *T. brusei s.l.* can establish co-infections with *T.b. rhodesiense* resulting in sick and debilitated animals being sold at the market by local farmers. And although animals are required to be treated before sale at their point of origin, a confounding variable is the decentralization of public services. Many animals are sold without being treated or are treated inappropriately. A total of 428 HAT cases occurred between December 1998 and April 2004 according to Serer Health Centre figures with seasonal peaks occurring three to six months after the wet season when tsetse are more prolific. Of these figures, 103 were early stage, 287 were late stage and 38 were not staged. Late stage constituted 67% of all staged cases indicating that early detection was lacking by the local health system. Hospital mortality rates were reported as being 4% (18/428).

One excellent point of the study is that Fèvre et al (2005) utilized a model to "estimate the proportion of undetected *T.b. rhodesiense* cases" based on an early to late ratio in Soroti District. The results showed unreported cases equaling 299 or 0.7 for each reported case with 95% CI of 170 – 438 or 0.4 – 1.02 respectively between December 1998 and April, 2004. Fatality rates for untreated cases would equal 100%.

Central point sampling from cattle in livestock markets in areas of human sleeping sickness.

Again Fèvre and his team looked at cattle "harbouring human infective parasites" with outbreak and spread of HAT resulting from cattle movement. Untreated animals from HAT endemic areas and sold at market in naïve areas is a major confounder. Prior to widespread livestock control programs, Fèvre et al (2006) tested the "hypothesis that livestock markets could be used as central point sampling locations representative of the surrounding area." A cross-sectional study utilized 50 cattle blood samples taken from each major livestock market in Soroti, Tororo and Kamuli Districts and from four to six arbitrarily chosen villages surrounding the markets. All animal samples were collected on

the same sampling day. This would produce an expected prevalence of 4% for *T. brucei* s.l. at a 95% CI.

The results showed that PCR determined prevalence for *T. brucei s.l.* were significantly higher in the Soroti epidemic district vs. the endemic Tororo and Kamuli districts. There were no major differences within Soroti District in regards to the prevalence of *T. brucei s.l.* The market prevalence was representative of the prevalence in surrounding villages. This was not the case for Tororo and Kamuli districts because market results demonstrated a higher proportion of infected animals. This study suggests that central point sampling at markets can be used to determine the prevalence of HAT infections during emerging epidemics (and not within endemic populations). Fèvre et al (2006) are careful to note that central point sampling of cattle should not replace actual samples taken from human subjects. Further studies are needed in endemic areas to improve understanding of persistent infections in reservoir animals to elicit efficient interventions.

One detractor of this study is that specific testing for *T.b. rhodesiense* was not conducted in the field. A simple microscope can identify blood protozoa, however, the SRA gene marker for *T.b. rhodesiense* can be detected with PCR tests. The authors operated on the premise that the prevalence of *T.b. brucei*:*T.b. rhodesiense* is constant at 3:1 and indicates the prevalence of *T.b. rhodesiense*, the organism that causes HAT. Similarly, *T.b. brucei* s.l. surveillance (although not pathogenic to animals) is indicative of more pathogenic cattle trypanosomes *T. vivax* and *T. congolense*. Again the author mentions that the more debilitated animals are sold at market and are brought back on subsequent market days if not sold. This leads to a significantly high build up of infected animals as tsetse blood meals on cattle will occur more frequently at the markets. So even if cattle arrived uninfected, chances are that they would soon become infected. This alone presents a confounding problem. However, the market is the ideal place to implement adequate control measures.

Domestic animals as reservoirs for sleeping sickness in three endemic foci in southeastern Uganda.

Persistent unpublished cases of HAT were reported in 2002 in southeast Uganda prompted researchers to look at reservoir hosts other than cattle. Wild animals aside, pigs, goats, sheep and dogs are domestic animals that live in close association with humans and the authors of this paper believe that local HAT transmission cycles are occurring in animals that receive less attention than cattle. Between March and August, 2000, venous whole blood was collected from 3,344 cattle, 123 goats, 106 sheep, 1181 pigs and 149 dogs. Animals were sampled from three agro-ecological zones in southeast Uganda - Kamuli (Zone I), Mukono (Zone II) and Tororo (Zone III).

Each microscopically diagnosed *T. brucei* positive sample was inoculated into two mice. Blood samples were drawn from each mouse between and inclusive of days one and 60 and preserved to be tested in blood incubation infectivity tests (BIIT) for sensitivity to human serum. Cattle and pigs showed the highest overall point prevalence in all three zones at 13.25% and 17.5% respectively. Four sheep in zone III only had a 3.8% overall point prevalence and no trypanosomes were found in goat or dog blood. A total of 443 positive cattle results showed 43% were infected with *T. brucei* and was similar in all three zones. And, 83.1% of the 207 trypanosome positive pigs were affected with *T.*

brucei. Parasitic isolates totaling 58/189 or 30.7% were human serum-resistant (HSR) indicating the human-infective *T. b. rhodesiense* organism. Isolates in cattle were 29.6%, pigs 30.2% and the sheep isolate of *T. brucei* was HRS.

Of note in this paper is the lack of data regarding HAT infections to verify the associations between humans and domestic animal reservoirs. This is a little disappointing. However the authors were able to suggest that HAT infections existed due to the presence of HSS or human-serum-resistance (HSR) of trypanosomes found within the different animal groups. It is inferred that HAT is very closely associated with animals as reservoir hosts. And reservoir hosts have persisted or infections have reoccurred due to the inability of drugs to penetrate certain body tissues where trypanosomes may reside e.g. brain tissue. Another point is that because porcine trypanosomiasis has not been studied in depth and clinical pigs appear healthy, little if any "curative or prophylactic" trypanocidal drugs exist for swine. And, pigs are probably major reservoir host of trypanosomiasis.

TRANSMISSIBILITY OF TRYPANOSOMA BRUCEI DURING ITS DEVELOPMENTS IN CATTLE.

A case-control study was conducted by Van den Bossche et al (2005) to further demonstrate the important role that cattle play in HAT resulting from *T. b. rhodesiense* infections. Stock male *Glossina morsitans morsitans* tsetse flies, less than 32 hours old were given a blood meal from anesthetized T. b. brucei or *T. congolense* infected mice. Approximately 40 flies were kept in cages for 30 days and maintained on three blood meals a week on rabbits. The rabbits were replaced once a week to avoid cyclical transmission. Eight susceptible (previously unexposed to trypanosomes) Friesian steers approximately six months of age, housed in a fly-proof stable were inoculated with *T. congolense*. After 26 days, all animals demonstrated high parasitemia, blood packed cell volume (PCV – a measure of anemia) less than 18% and fevers if their body temperature was greater than 39°C were treated with diminazene aceturate. After 30 days, once the PCV normalized all animals were exposed to tsetse flies on their flanks infected with *T. b. brucei*. After three months, the cattle were challenged with *T. congolense* infected tsetse flies resulting in a mixed *T. b. brucei* and *T. congolense* infection. All animals had blood samples drawn three times per week to determine PCV and parasitemia levels and were treated with diminazene aceturate at the end of the experiment. Any changes in parasitemia were determined by counting the number of parasites in 250 microscopic fields total.

A total of 1,192 flies were dissected with 348 (29.2%) having developed procyclic midgut infection and 100 developed metacylic infection. Infection rates were determined via intrinsic vectorial capacity (IVC). IVC = p X m where p = proportion of flies with procyclic infection in their midgut and m = proportion of flies with metacyclic infection in their salivary glands. Statistical analysis was performed using cross sectional logistic regression. High significance was determined during the acute phase and mixed *T. b. brucei* and *T. congolense* infection than in the chronic phase of infection. Acute phase is defined with parasitemia, increase body temperatures and decreased PCV. Chronic phase is defined with low normal stabilized PCVs and no fevers and parasitemia decreases. Once chronic phased animals are challenged with *T. congolense*, *T. brucei* para-

sitemia and PCVs increase. This implies that there are a significant number of trypanosomes to infect tsetse flies. This information can extrapolated in that chronically infected animals exposed to *T. b. rhodesiense* will have an increase in parasitemia and PCVs. Thus a "...high transmission rate during the various phases of infection combined with close proximity between people and cattle can lead to human sleeping sickness." But the authors state that this study "...gives no additional cues on the factors which generate either an epizootic or an endemic sleeping sickness.

Once again, this paper maintains that *T. b. rhodesiense* infected cattle are targets for control of HAT. Treatment with trypanocidal drugs (e.g. diminazene aceturate) will reduce the cattle reservoir host. However, one needs to be cautious in that if drugs are used inappropriately, this confounding problem may result in resistance in trypanosomes and a perpetuation of HAT. "Control of the tsetse population without addressing the parasite's reservoir will only result in a temporary solution".

CONCLUSION

In this search of the literature, not many studies exist describing the actual prevalence rates of HAT disease as it relates to the presence of trypanosomes in cattle. There is anecdotal evidence that supports the theory that if cattle (including pigs and sheep) are infected with trypanosomes, HAT epidemics are occurring or will occur. And, that if HAT epidemics are occurring, then it is probably due to an increase in reservoir numbers. Pigs are just as important a reservoir host if not more than cattle for *T. brucei* subgroups. That porcine trypanosomiasis has received a great deal less attention than bovine trypanosomiasis. And, that there is "little if any curative or prophylactic treatments of pigs with trypanocidal drugs (partly because infected pigs often appear healthy according to Waiswa et al (2003).

Uganda is the only African nation with both *T. b. rhodesiense* and *T. b. gambiense* endemics. And, as of April 2004 there are reports of HAT cases in Kaberamaido District, northwest of Soroti District and some reports in Lira District to the north of Kaberamaido. There have been 144 cases reported between February 2004 and January 2005 from these areas. Very sick emaciated and infected animals were probably sold at markets for cheaper prices and if they could make the journey probably ended up in the poorer Kaberamaido and Lira districts causing the spread of the HAT epidemic. As a result, there is a slow movement of *T. b. rhodesiense* toward the *T. b. gambiense* endemic area of Uganda. If geographical overlap of these two HAT occurs, there will be significant problems arising as the diagnosis and treatment methods for humans and animals are different. Prevention is more effective than curative measures, but civil unrest may be the downfall of any attempts by government agencies.

The above situation in Uganda alone is one reason why there is an urgent need for more studies involving trypanosome infection in cattle and HAT in addition to the multitude of studies being conducted on the tsetse fly and the trypanosome. It is not just enough to control the tsetse fly vector. Most of the researchers in the papers discussed here agree that "Control of the tsetse population without addressing the parasite's reservoir will only result in a temporary solution." Van den Bossche, et al (2005). This is due primarily to the persistent infections that exist in cattle and swine reservoirs.

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