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Impact of mass chemotherapy in domestic livestock for control of zoonotic *T. b. rhodesiense* human African trypanosomiasis in Eastern Uganda

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ABSTRACT

Introduction: Human African trypanosomiasis (HAT) comprises two fatal parasitic diseases. Uganda is home to both chronic *T. b. gambiense* (gHAT) and the acute zoonotic form *T. b. rhodesiense* (rHAT) which occur in two large but discrete geographical foci. The area affected by rHAT has been rapidly expanding due to importation of *T. b. rhodesiense* infected cattle into tsetse infested but previously HAT free districts. Migration of rHAT has resulted in a considerable human health burden in these newly affected districts. Here, we examined the impact of a single, district-wide, mass chemotherapeutic livestock intervention, on *T. b. rhodesiense* prevalence in cattle and on incidence and distribution of human rHAT cases in Kamuli and Soroti districts in eastern Uganda.

Methods: A single mass intervention in domestic cattle (n = 30,900) using trypanocidal drugs was undertaken in November and December 2002 under the EU funded Farming in Tsetse Controlled Areas (FITCA) programme. The intervention targeted removal of the reservoir of infection i.e. human infective *T. b. rhodesiense* parasites in cattle, in the absence of tsetse control. Interventions were applied in high-risk sub-counties of Kamuli district (endemic for rHAT) and Soroti district (where rHAT has been recently introduced). The prevalence of *T. brucei* s.l. and the human infective subspecies, *T. b. rhodesiense* in cattle (n = 1833) was assessed before and 3 and 12 months after intervention using PCR-based methods. A combination of descriptive statistical analysis and spatial scan statistics were applied to analyse rHAT cases reported over a 5-year period (January 2000–July 2005).

Results: A single intervention was highly effective at removing human infective *T. b. rhodesiense* parasites from the cattle reservoir and contributed to a significant decrease in human rHAT cases. Intervention coverage was higher in Kamuli (81.1%) than in Soroti (47.3%) district but despite differences in coverage both districts showed a reduction in prevalence of *T. b. brucei* s.l. and *T. b. rhodesiense*.

In Kamuli, the prevalence of *T. brucei* s.l. decreased by 54%, from 6.75% to 3.11%, 3 months post-intervention, rising to 4.7% at 12 months. The prevalence of *T. b. rhodesiense* was 3% pre-intervention and no *T. b. rhodesiense* infections were detected 3 and 12 months post-treatment. In Soroti, the prevalence of *T. brucei* s.l. in cattle decreased by 38% (from 21% to 13%) 3 months after intervention decreasing to less than 10% at 12 months. The prevalence of *T. b. rhodesiense* was reduced by 50% at 12-months post-intervention (6%–3%). Most notably, was the impact of the intervention on the population dynamics between *T. b. brucei* and human infective *T. b. rhodesiense*. Before intervention in Kamuli district 56% of *T. b. brucei* s.l. circulating in cattle were *T. b. rhodesiense*; at both 3 and 12 months after intervention none of the re-infecting *T. b. brucei* s.l. were human infective, *T. rhodesiense*.

For human rHAT cases, there was a seven-fold decrease in rHAT incidence after intervention in Kamuli district (5.54 cases/1,000 head of population 2000–2002 to 0.76 cases/1,000, 2003–2005). Incidence data suggests that the intervention had minimal impact on the number of rHAT cases in Soroti overall, but showed a significant decrease in the seasonal peak of cases in the year following treatment.

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Conclusion: A single intervention, targeted at cattle, introduced at district level, in the absence of tsetse control, was highly effective at removing human infective rHAT parasites from the cattle reservoir and contributed to a significant decrease in human rHAT cases. The differential impacts observed between the two districts are related to both the different stages of rHAT endemicity in the districts, and levels of intervention coverage achieved in the cattle population. Treatment of cattle to remove the reservoir of rHAT infection offers a promising and cost effective approach for the control of rHAT. It is important that cattle are treated before relocation to prevent possible merger of the two HAT foci, which would complicate diagnosis and treatment of both gHAT and rHAT.

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1. Introduction

Human African Trypanosomiasis (HAT) or sleeping sickness is a neglected tropical disease (NTD) transmitted by tsetse flies (*Glossina* spp.). HAT comprises two distinct diseases caused by two sub-species of *T. brucei* s.l. *Trypanosoma brucei gambiense* and *T. b. rhodesiense* (Welburn et al., 2001a). *T. b. gambiense* HAT (gHAT) is a chronic disease passed between humans by human-tsetse-human contact and sustained by vertical transmission (Wastling et al., 2011; Welburn et al., 2016a). *T. b. rhodesiense* HAT (rHAT) is an acute disease with a complex zoonotic epidemiology involving tsetse transmission between a range of wildlife, livestock reservoirs and humans (Anderson et al., 2011). Both forms of HAT are fatal if untreated with death occurring 6–8 months after infection with rHAT and around three years after first indication of gHAT infection (Checchi et al., 2008) although patients have been shown to carry gHAT infections for decades (Fèvre et al., 2005; Welburn et al., 2016b).

Uganda is unique in having active foci of both forms of HAT which have co-existed in Uganda for over a century (Keorner et al., 1995; Fèvre et al., 2004) in discrete geographical foci; gHAT in the northwest and rHAT around the shores of Lake Victoria in south-eastern and central regions (Welburn et al., 2001a, 2016a).

Following a large outbreak in Tororo district in 1987 (Odiit et al., 2004) rHAT has progressively migrated around the shores of Lake Kyoga (Batchelor et al., 2009). Domestic cattle have been shown to be the major reservoir of *T. b. rhodesiense* in Uganda (Welburn et al., 2001a,b) and importation of infected cattle from districts in established rHAT has caused rHAT outbreaks in previously unaffected districts (Fèvre et al., 2001) moving rHAT towards the gHAT focus (Picozzi et al., 2005).

In 1999 rHAT first emerged in Soroti district (Fèvre et al., 2001) and was subsequently introduced into Kaberamaido and Dokolo districts (Batchelor et al., 2009) and other districts bordering Lake Kyoga (Apac and Amolatar) and to the northern district of Lira (Wardrop et al., 2012, 2013). In Soroti, rHAT spread from the point of introduction, Brooks Corner Cattle market (Fèvre et al., 2001), throughout the larger part of the district, the district reporting more than 500 cases (Batchelor et al., 2009). Cattle importation was fuelled by a number of major cattle restocking programmes that aimed to improve rural livelihoods (Selby et al., 2013). The rapid expansion of the area affected by rHAT towards the gHAT endemic focus is a major public health issue in Uganda. When the two disease overlap, diagnosis and treatment will be compromised with a significant impact on human morbidity and mortality (Welburn and Coleman, 2015).

Domestic cattle constitute the major reservoir of *T. b. rhodesiense* infection (Welburn et al., 2001a,b) in Uganda and given the predisposition of tsetse flies to feed on cattle, it was predicted that treatment of 85% of cattle with a single dose of trypanocide (a veterinary anti-parasitic treatment) would interrupt rHAT transmission to humans in Uganda (Welburn et al., 2006).

In 2000, Farming in Tsetse Controlled Areas (FITCA) a large European Union funded agricultural development project undertook a survey of rHAT and African Animal Trypanosomiasis (AAT) across 12 districts of Uganda (FITCA, 2005). Two districts, Kamuli and Soroti, which showed high rHAT incidence were selected for mass administration of a single dose of trypanocidal drug, aimed at reducing the prevalence of *T. b. rhodesiense* infection in domestic cattle. Administration of the trypanocidal treatment impacts on rHAT and all AAT parasites.

Multiple species and sub-species of trypanosomes circulate in cattle within HAT foci, including those that impact on animal health and cause AAT. While *T. congolense* and *T. vivax* are pathogenic to livestock, impacting on both animal health and productivity across much of Uganda (Okello et al., 2015); *T. b. brucei* (not infective for humans) and *T. b. rhodesiense* cause only mild illness in indigenous breeds with infection often undetected. *T. b. brucei* and *T. b. rhodesiense* are however, extremely pathogenic to exotic cattle (Wellde et al., 1989) and prevent upgrading of stock.

Kamuli district, lies within the Busoga HAT focus, and has been endemic for rHAT since at least the 1980's (Mbulamberi, 1989). In 2001, Kamuli reported over 100 HAT cases. *T. b. rhodesiense* HAT only emerged in Soroti district in December 1998. A single rHAT case was previously in Soroti in the 1960s, but the patient was considered to have been infected elsewhere, while travelling to Tanzania, passing through rHAT endemic foci (Onyango, 1967). A survey conducted in Soroti at that time failed to identify any *T. brucei* s.l., in humans or animals (Mwambu, 1969).

Here we make a comparative analysis of the impact of district level administration of a single dose of trypanocide in Kamuli, an established endemic rHAT focus and in Soroti, a newly affected focus, undertaken by FITCA in 2002. We examined the prevalence of *T. brucei* s.l. and *T. b. rhodesiense* in cattle before treatment and at 3 and 12 months after treatment. We also examined the impact of the intervention on the relationship between the human and non-human infective subspecies of *T. brucei*. To assess the impact of the intervention on the incidence of reported human sleeping sickness cases, we analysed the number, distribution and incidence of rHAT cases in the districts between January 2000 and July 2005.

2. Material and methods

2.1. FITCA intervention

Kamuli and Soroti districts lie on the southern and northern shores of Lake Kyoga respectively (Fig. 1a). An estimated 98% of the population of Kamuli and 89% of the population of Soroti are engaged in subsistence agriculture (Ugandan Bureau of Statistics, 2002). Within each district, FITCA designated 3 sub-counties as high-risk for rHAT: Pingire, Kateta and Kyere in Soroti, and Bumanya, Kitayunjwa and Namwendwa in Kamuli. High-risk areas were sub-counties where rHAT had been reported between 1996 and 2001. Medium risk areas showed AAT levels >5% (identified by microscopy) but no HAT cases. Low-risk areas had levels of

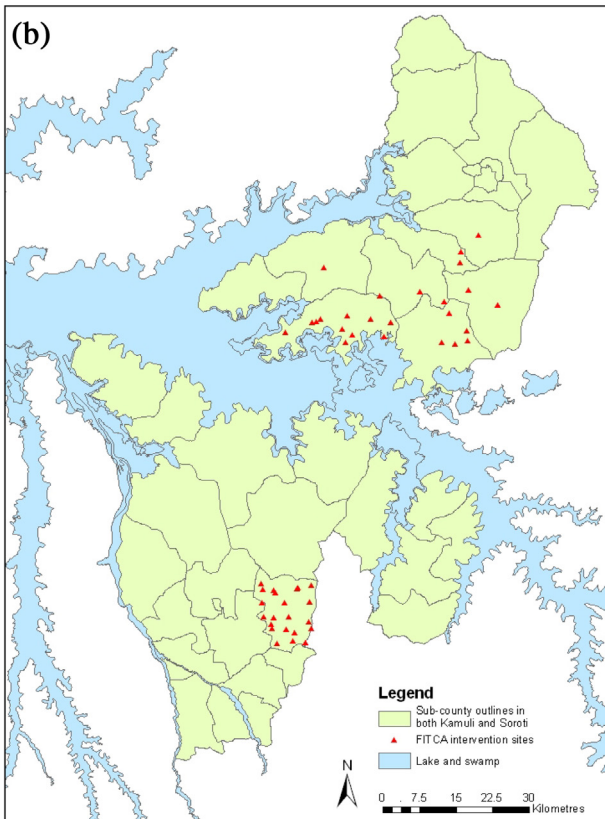
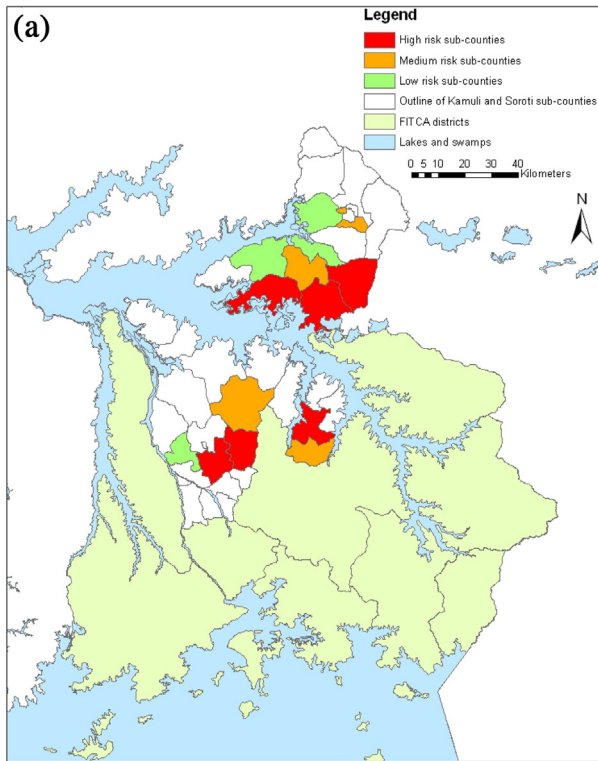


Fig. 1. (a) Sub-counties considered at high, medium and low risk for rHAT in Kamuli and Soroti districts (other FITCA districts pale green). (b) Intervention sites in Soroti and Kamuli districts. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

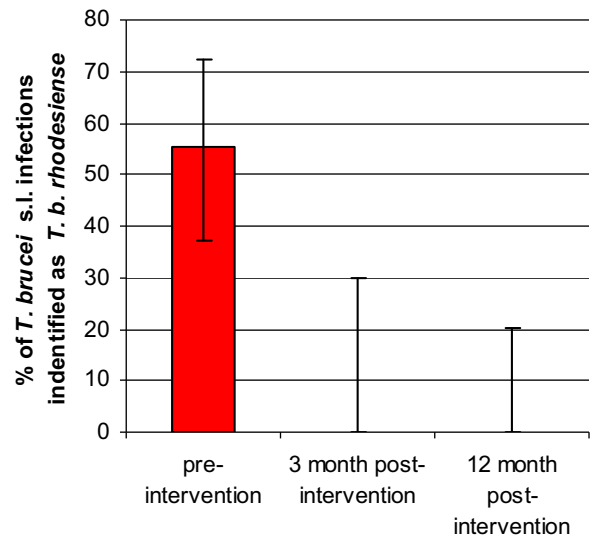
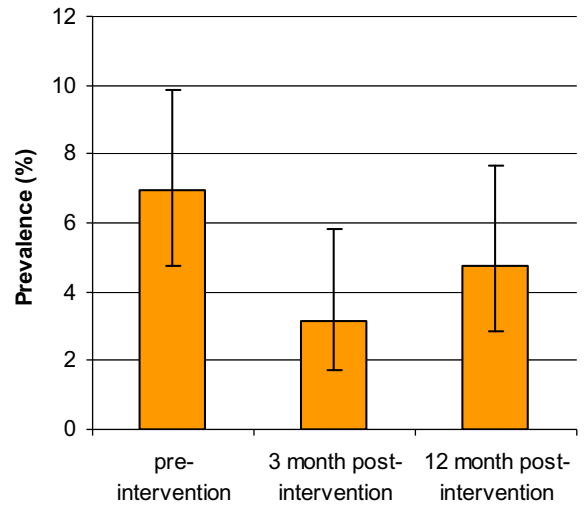


Fig. 2. (a) Prevalence of *T. brucei* s.l. (yellow) in Kamuli at each time point (Samples examined: Pre-intervention = 400; 3m = 289; 12m = 319); and (b) *T. b. rhodesiense* (red) as percentage of the total *T. brucei* s.l. identified (number of *T. brucei* s.l. samples examined for SRA gene/human infectivity; Pre-intervention = 27; 3m = 9; 12m = 15). Error bars represent 95% binomial confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

AAT of <5% and no human cases. Areas with no evidence of AAT or where no data was collected were given a no risk status. (Fig. 1a). Within the high-risk areas, FITCA administered a single dose of isometamidium chloride (ISMM) at 0.5 mg/kg or a single dose of diminazene aceturate (DIM) at 3.5 mg/kg to animals with clinical signs of AAT. ISMM was preferentially applied for its prophylactic properties, the drug remaining active in the animal bloodstream 3 months (Holmes et al., 2004). No tsetse traps were deployed during the intervention within the study area.

The trypanocidal drug intervention was undertaken in 9 sub-counties, seven in Soroti and two in Kamuli district. In Soroti, 25 intervention sites were selected across Bugondo, Serere, Pingire, Kateta, Kyere, Asuret and Atiira sub-counties (Fig. 1b). In Kamuli, 22 intervention sites were selected; 21 in Namwendwa sub-county and a single site in Kiatyunjwa sub-county (Fig. 1b). In total 30,900 cattle were treated: 11,900 in Soroti and 19,000 in Kamuli. In the

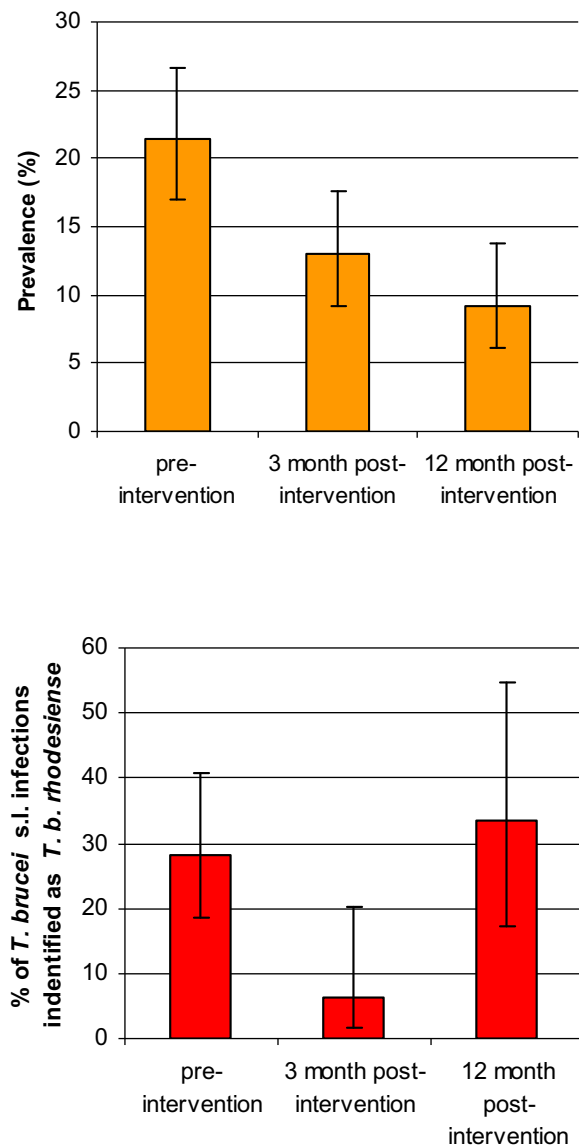


Fig. 3. (a) Prevalence of *T. brucei* s.l. (yellow) in Soroti at each time point (Samples examined: Pre-intervention = 290; 3m = 256; 12m = 240); and (b) Proportion of *T. brucei* that is human infective *T. b. rhodesiense* (red) before and after a single trypanocidal administration in Soroti district as a percentage of the total *T. brucei* s.l. identified (*T. brucei* s.l., examined for SRA gene pre-intervention = 60; 3m = 32; 12m = 21). Error bars represent 95% binomial confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

absence of data from a reliable livestock census it was difficult to gauge the proportion of cattle covered in these intervention sites and to estimate coverage, farmers were asked when their animals were sampled whether their cattle had participated in the intervention.

2.2. Cattle sampling

Blood samples were collected at the point of trypanocidal administration in November 2002, and at 3 and 12 months after intervention. Sample sizes were calculated using a livestock census undertaken by FITCA. Farmers presented their animals at a central point for trypanocidal drug administration and 51–56 animals were randomly sampled at each site. After intervention, cattle owners who presented their animal for sampling were asked whether that animal was previously treated under the FITCA intervention. A cat-

tle de-wormer application was offered to farmers as an incentive for their participation. One hundred microliters of blood, was drawn from the ear vein using a sterile lancet, and collected in two 50 μ l heparinised capillary tubes and applied directly to an Whatman® FTA® classic card. FTA® cards were left to dry at room temperature for 24 h and stored in airtight pouches with desiccant.

In total, 1,803 cattle were sampled; 1,017 from Kamuli and 786 from Soroti (Table 1). Cattle were sampled from 5 high-risk rHAT villages (3 in Kitayunjwa sub-county in Kamuli and 2 in Pingire sub-county in Soroti District). The average number of cattle sampled at each time point was 339 in Kamuli (7.7% of the cattle population of Kitayunjwa sub-county, estimated at 4,425) and 262 in Soroti (3.5% of the cattle population in Pingire sub-county, estimated at 7,589).

2.2.1. PCR analysis

The prevalence of *T. brucei* s.l. (non-human infective *T. b. brucei* and human infective *T. b. rhodesiense*) in cattle was determined using species and sub-species specific primers. Samples positive for *T. brucei* s.l. were subsequently tested for the presence of the *T. b. rhodesiense* SRA gene, conserved within all *T. b. rhodesiense* parasites isolated in Uganda (Welburn et al., 2001b) using a multiplex PCR reaction (Picozzi et al., 2008), which can discriminate the two subspecies. Five discs (diameter 3 mm) were taken from each Whatman® FTA® card sample and processed as described in Picozzi et al. (2008). In brief, discs were washed twice with FTA purification reagent and twice with TE buffer, before being air dried. Washed discs were used to seed 5 separate PCR reactions per sample. If any reaction was positive the sample was deemed positive. PCR reaction conditions, primer sequences and adapted cycling conditions were as described in Picozzi et al. (2008). PCR products were visualized by electrophoresis on a 1.5% agarose gel using GelRed DNA stain and gels documented with a BioRad GelDock™ imaging system.

Prevalence of *T. brucei* s.l. and *T. b. rhodesiense* as detected by PCR was expressed as a percentage, and exact binomial 95% confidence intervals were computed (R, version 2.4.0). χ^2 tests were conducted in Minitab version 14 (Minitab, Inc.). Differences were considered to be significant at $p < 0.05$. Paired *t*-tests were considered to be significant at $p < 0.05$.

2.3. rHAT case data

Statistical analysis and spatial scan statistics were used to analyse rHAT case data for Kamuli and Soroti provided to the FITCA project, between January 2000–July 2005. HAT diagnoses were made using WHO criteria of visual detection of trypanosomes in blood or cerebrospinal fluid (Acup et al., 2016). Data available included village, parish and sub-county of residence, admission date and disease and stage. Data were digitised and stored in Excel. For Kamuli, rHAT data was reported from diagnostic facilities in Bulopa, Nankandulo and Namwendwa. HAT cases from Soroti District were recorded at Serere Hospital, the district diagnostic facility (Acup et al., 2016).

T. b. rhodesiense HAT (rHAT) incidence was calculated using the 2002 census data for sub-county and parish populations (Ugandan Bureau of Statistics, 2002). Data were analysed year on year with comparisons made in the period before administration of trypanocides (January 2000–December 2002) and after (January 2003–July 2005) drug intervention.

The spatial scan statistic (Kulldorff, 1997) was applied for spatial clustering of HAT and implemented using SaTScan version 5.1.3 Analysis assumed a Bernoulli distribution. Paper based maps of the study areas were scanned and geo-referenced using image warp version 2.0 for ArcView version 3.1 Geographical Information System (GIS) software (ERSI Systems, Redlands, CA, USA). 1:50,000 maps were digitised and geo-referenced for (i) Kamuli; Balawoli (Y732, 52/3,1-U.S.D. 1963), Kamuli (Y732, 62/1, 3-U.S.D.

Table 1
Numbers of cattle sampled, pre-intervention and 3 and 12 months post-intervention.

District	Village	Pre intervention	3 months post-intervention	12 months post-intervention	Total (n)
Kamuli	Bulagala	120	63	108	291
	Buwaiswa	143	126	101	370
	Nabigoagerya	146	100	110	356
Soroti	Amuria	146	120	120	386
	Okidi	144	136	120	400
Total		699	545	559	1,803

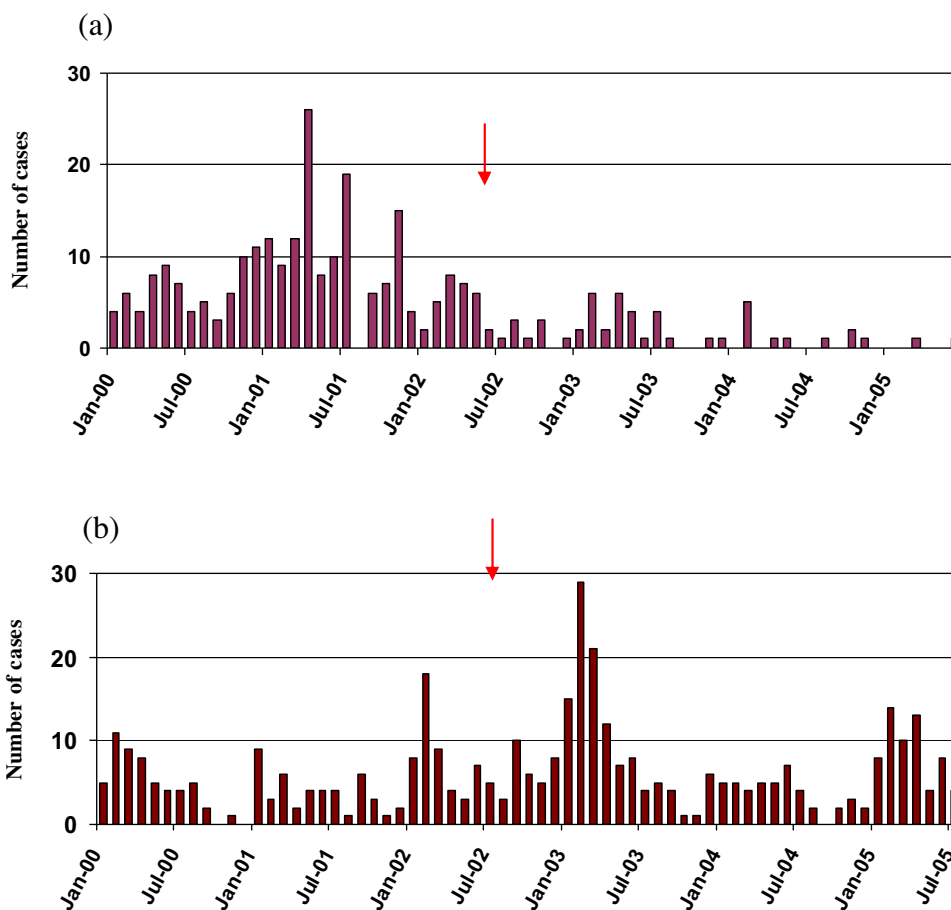


Fig. 4. Monthly *T. b. rhodesiense* HAT cases reported January 2000 to July 2005 in (a) Kamuli district and (b) Soroti districts. Red arrows indicate intervention point. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1998), Namwendwa (Y732, 62/2, 3-U.S.D. 1998) and Nawaikoke (Y732, 52/4, 1-U.S.D. 1963) and (ii) Soroti; Bugondo (Y732, 42/4, 1-U.S.D.1963), Kelle (Y732, 42/3, 1-U.S.D. 1964), Kumi (Y732, 53/2, 1-U.S.D. 1963), Kyere (Y732, 53/1, 1-U.S.D. 1964), Sambwa (Y732, 52/2, edition 1-U.S.D. 1969) and Soroti (Y732, 43/3, 1-U.S.D. 1968).

Village locations and GIS co-ordinates were recorded on the digital maps. Crush pen co-ordinates for intervention site were recorded using hand held Garmin GPSII+ devices. Mann Whitney *U* tests (Minitab version 14) were applied to compare median, monthly HAT cases/year (p value of <0.05 was considered significant). Villages with single or multiple HAT cases were defined ‘case villages’, villages that did not report a HAT case during the study period were defined as ‘control villages’. A maximum cluster size was set at 50% dataset points and analysis ran to identify areas of high rates of clustering (Kulldorff and Nagarwalla, 1995; Kulldorff et al., 1998) with 9999 Monte-Carlo iterations. Clusters with *p*-values <0.05 were considered significant.

3. Results

3.1. Prevalence of *T. brucei* s.l. and *T.b. rhodesiense* in cattle before and after trypanocidal intervention in Kamuli and Soroti districts

In Kamuli district, the *T. brucei* s.l. prevalence in cattle (n = 1017) pre-intervention was 6.75%, over half (56%) of which were human infective (*T. b. rhodesiense*). The prevalence of *T. b. rhodesiense* was 3% before intervention. Three months after drug administration *T. brucei* s.l. prevalence reduced to 3.11% and no human infective *T. b. rhodesiense* were identified in cattle. Twelve months after intervention the prevalence of *T. brucei* s.l. remained lower than before drug administration (4.7%) and no human infective parasites were detected (Fig. 2a and b).

In Soroti district, the *T. brucei* s.l. prevalence in cattle before intervention was 21%, three times greater than that observed in Kamuli (Fig. 3). The prevalence of *T. b. rhodesiense* prior to intervention

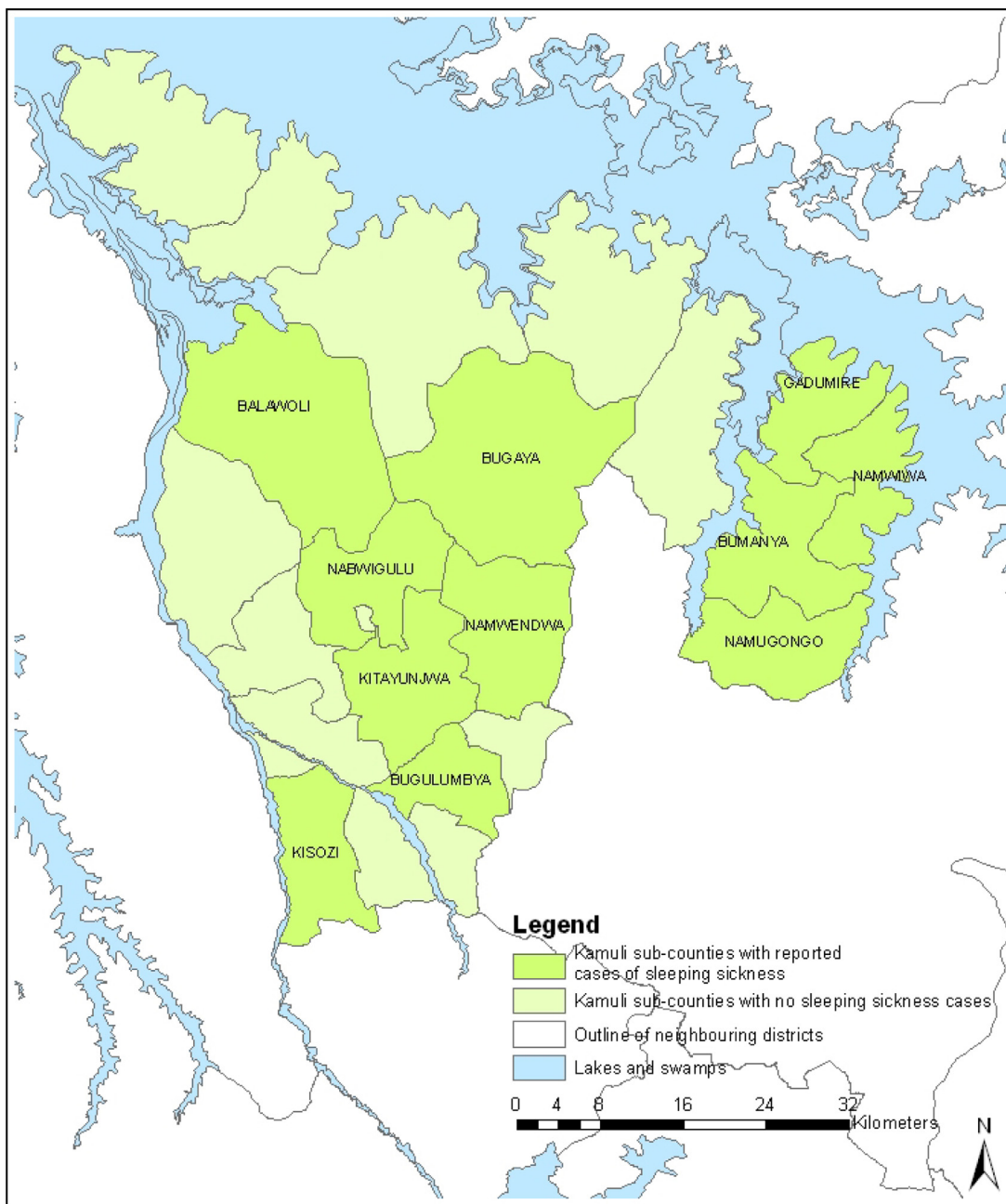


Fig. 5. *T. b. rhodesiense* HAT (rHAT) affected sub-counties in Kamuli district (January 2000–July 2005).

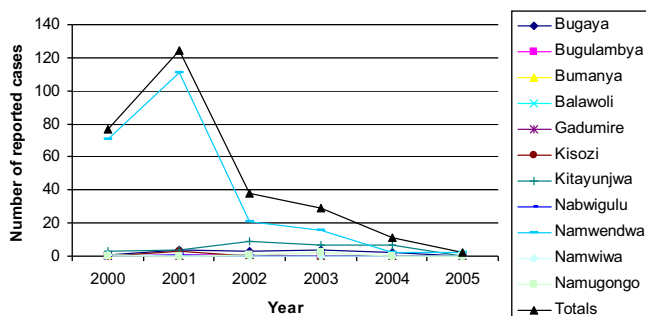


Fig. 6. *T. b. rhodesiense* rHAT cases in affected sub-counties of Kamuli district (January 2000–July 2005).

was 6%, twice that observed in Kamuli. However, the proportion of *T. brucei* s.l. infections that were human infective was lower at 28%.

The *T. brucei* s.l. prevalence was significantly reduced 3 months post intervention to 13% ($p < 0.01$) with less than 6% of these being human infective. The prevalence of *T. b. rhodesiense* reduced to 0.78%. Twelve-months post-intervention *T. brucei* s.l. prevalence was 10%, significantly lower ($p < 0.001$) than pre-intervention. The prevalence of *T. b. rhodesiense* was 3% – half that observed pre-intervention (Fig. 3a). However, 32% of the *T. brucei* s.l. were human infective *T. b. rhodesiense* ($p < 0.01$), see Fig. 3b.

3.2. Coverage of trypanocidal drug administration in Kamuli and Soroti districts

Intervention coverage may have influenced the impact of the mass drug administration on parasite prevalence. Higher number of farmers in Kamuli reported their animals having been given the trypanocidal drug (81.1% reported their animals receiving drug during the 3-month post-intervention sampling and 67.2% in the

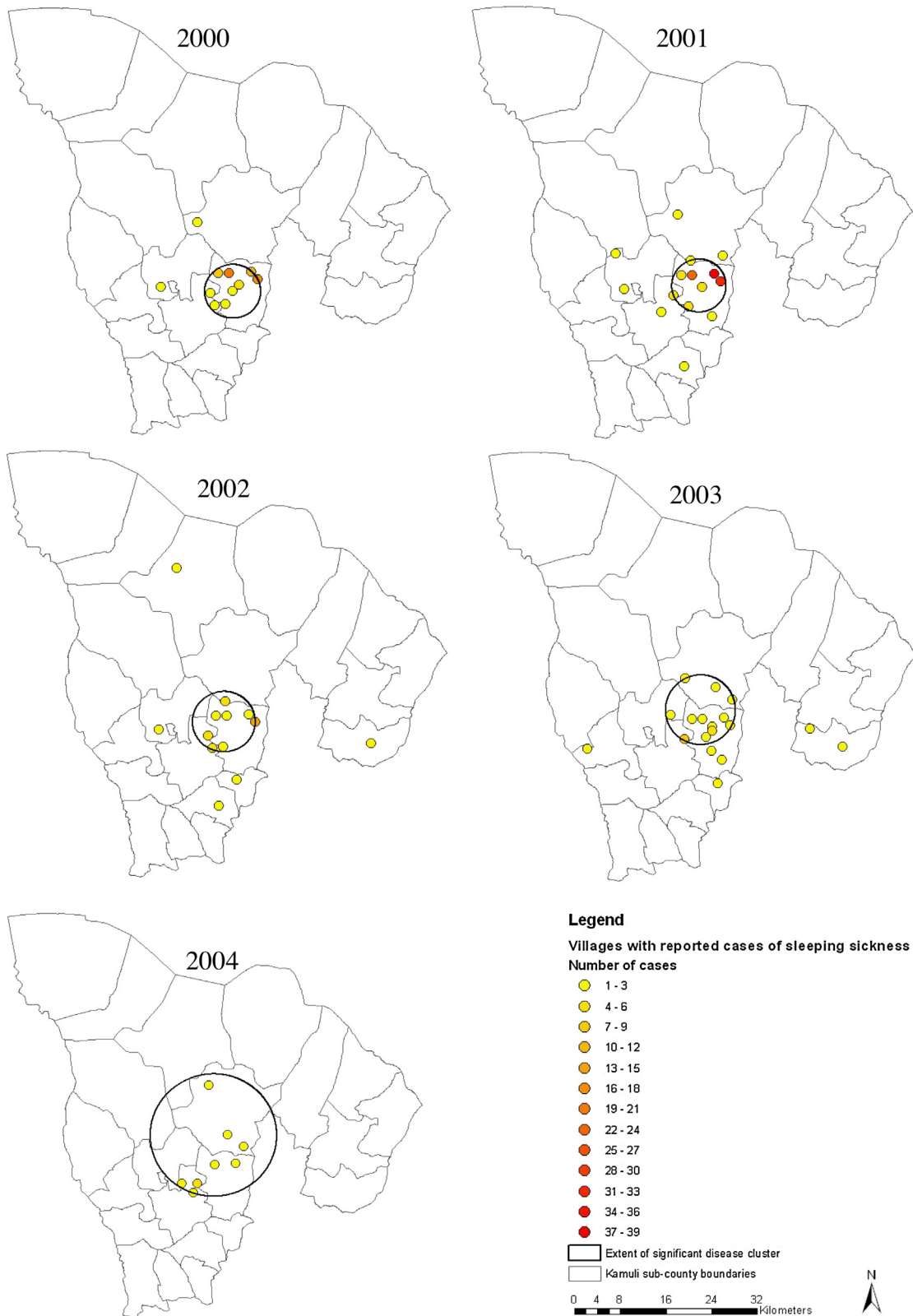


Fig. 7. *T. b. rhodesiense* HAT case distribution and significant disease clusters (circled) in Kamuli district (2000–2004).

12-month post-intervention sampling). Farmers reported lower coverage in Soroti from (47.5% during 3-month post-intervention sampling and 47.3% during the 12-month post-intervention sampling).

3.3. HAT cases reported in Kamuli and Soroti districts (2000–2005)

Between January 2000 and July 2005, 283 rHAT cases reported in Kamuli (Fig. 4a) and 403 rHAT cases were reported in Soroti (Fig. 4b).

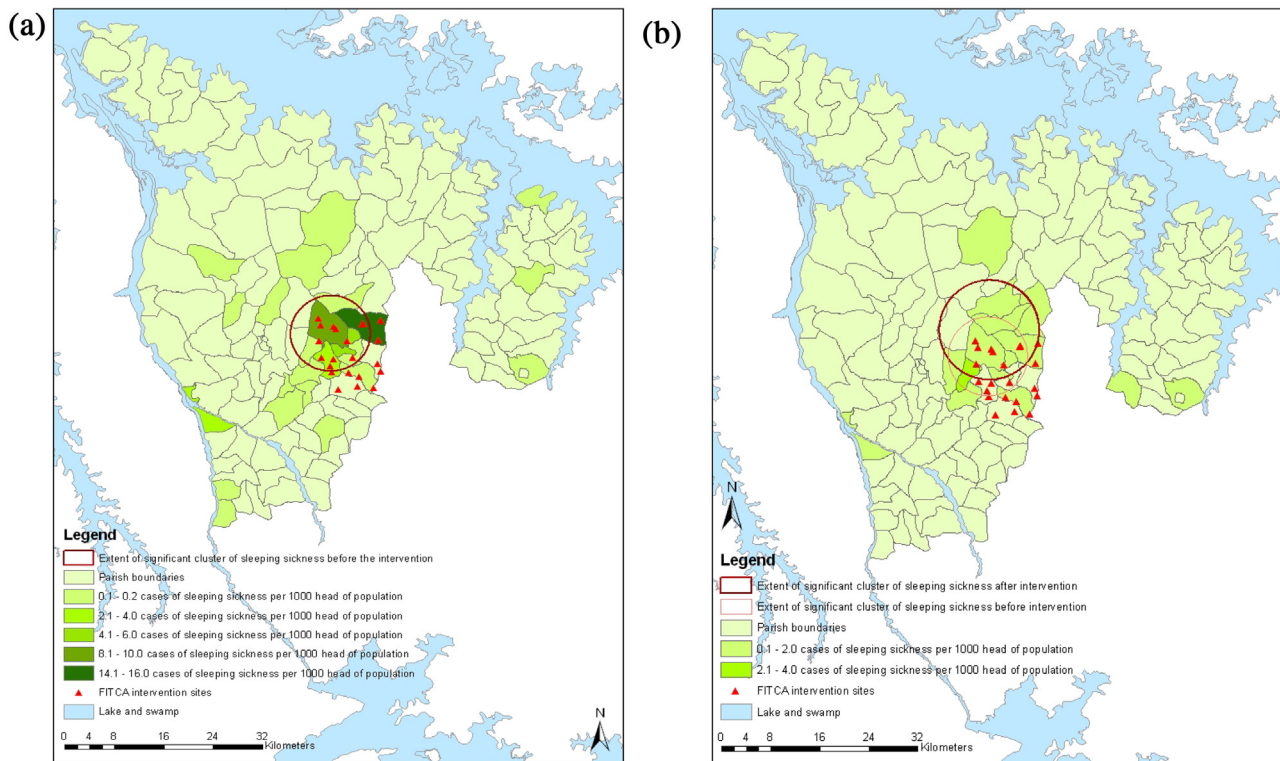


Fig. 8. *T. b. rhodesiense* HAT incidence per 1,000 head of population in Kamuli (a) pre- (January 2000–December 2002) and (b) post-intervention (January 2003–July 2005).

Table 2
Median monthly *T. b. rhodesiense* HAT cases reported in Kamuli and Soroti districts 2000–2005.

Year	Median monthly rHAT cases	
	Kamuli	Soroti
2000	6.5	4.5
2001	9.5	3.5
2002	2.5	6.5
2003	1.5	6.5
2004	0.5	4.0

Overall, the number of cases reported in Kamuli district during the study period were relatively stable. Elevated cases numbers were reported in April, July and October 2001 and from January 2000 to mid- 2002 (Fig. 4a). The median monthly number of rHAT cases each year was used to examine year-on-year change (Table 2). Post-intervention, Kamuli district reported a declining number of cases and a significant decrease in rHAT cases, was observed between 2001 and 2002 ($p=0.002$).

In Soroti district, reported rHAT cases showed seasonal variation, peaking in February/March, notably in 2002 and 2003 (Fig. 4b). The median number of monthly rHAT cases was relatively stable over the 5-year period (Table 2). Most rHAT cases are reported at late stage approximately 6-months after infection (Odiit et al., 2004; Acup et al., 2016). Cyclical development of *T. brucei* infections in tsetse takes 4–6 weeks, from feeding on an infected host to being able to transmit that infection back to another mammal (Welburn et al., 1995; Milligan et al., 1995) and so any impact of cattle treatment on human infection would not be visible within the case records until March 2003. The peak of rHAT cases observed in years 2000, 2002 and 2003 is notably absent in 2004 but returns in 2005 (Fig. 4b).

3.3.1. rHAT cases within Kamuli sub-counties

From January 2000 to July 2005, 283 rHAT cases were reported in Kamuli in 11 of 23 sub-counties (Fig. 5). Three sub-counties consistently reported rHAT cases, Namwendwa (all years) and Bugaya and Kitayunjwa (all but 2005). Namwendwa reported 79% of all rHAT cases in the Kamuli district; three villages (Kyeeya, Kinu, Ndalike and Isingo) consistently reported cases between 2000 and 2003 (Fig. 6).

In the nine parishes that hosted an intervention site there was a decrease in the incidence of reported rHAT cases from 5.54 cases per 1,000 head of population pre-intervention to 0.76 cases per 1,000 head of population, post intervention. Only Nawansaso parish showed an increase in rHAT cases post-intervention (Table 3).

A significant primary cluster of rHAT cases, 5.76–8.14 km radius was observed from 2000 to 2003 in northwest Namwendwa sub-county. This cluster increased to 13.85 km in 2004, migrating northwards into southern Bugaya (Figs. 7 and 8).

3.3.2. Spatial distribution of HAT cases pre- and post-intervention in Soroti district

Between January 2000 to July 2005, 403 rHAT cases were reported from 77 villages in 6 of 17 sub-counties in the south of Soroti district (Fig. 9). Asuret, Kateta, Kyere and Pingire reported cases annually and Serere/Olio reported cases in all years but 2004. Atiira reported cases in 2001 and 2004. The peak of rHAT cases in 2003 is due to the high number of cases from Pingire and Asuret (Fig. 10).

There was no significant difference in rHAT case incidence before (3.82 cases/1,000 people) and after intervention in Soroti district (4.50 cases/1000 people). There was no change in rHAT case incidence across the 14 parishes that hosted an intervention site (1.27 cases/1,000 pre-intervention and 1.59 cases/1,000 post-intervention). Where intervention sites were located; five parishes showed no change in rHAT incidence, four showed an increase and five a decrease post-intervention (Table 4).

Table 3
 Cases of *T. b. rhodesiense*HAT (rHAT) reported from parishes hosting an intervention site in Kamuli district (pre- and post-intervention). Reported rHAT cases from 9 parishes containing an intervention site are shown. Relative change is shown as ↓ for a decrease and ↑ for an increase in the number of reported cases.

Sub-county	Parish	Intervention sites	rHAT cases pre-intervention (Jan 00 to Dec 02)	rHAT cases post-intervention (Jan 03–July 05)	Change
Namwendwa	Kyeeya	4	44	4	↓
	Ndalike	4	109	5	↓
	Isingo	1	15	4	↓
	Namwendwa	1	6	0	↓
	Kidiki	2	0	0	–
	Bulange	3	0	2	↑
	Makoka	2	0	0	–
	Bulogo	4	14	0	↓
Kitayunjwa	Nawansaso	1	9	12	↑
Total		22	197	27	↓

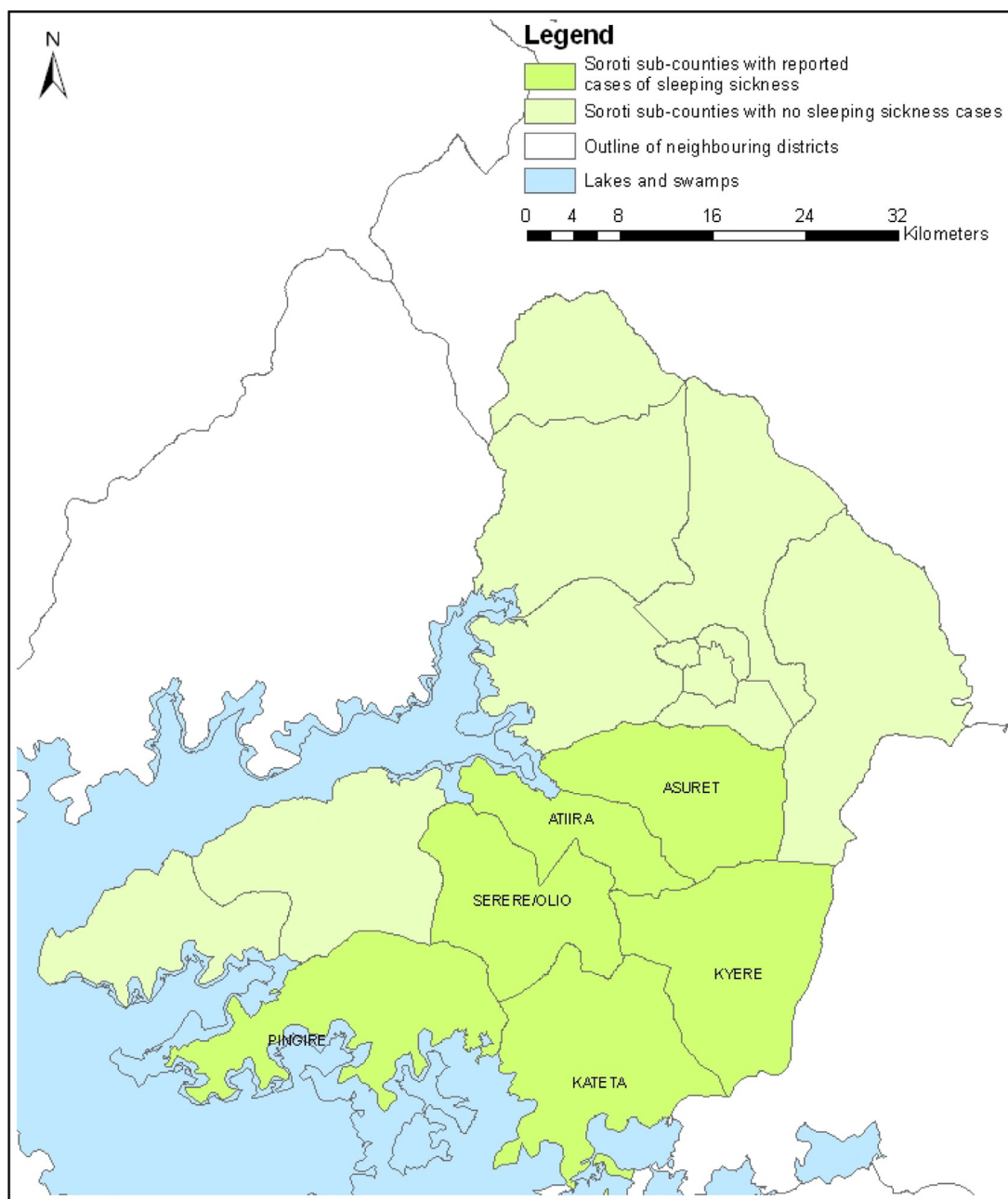
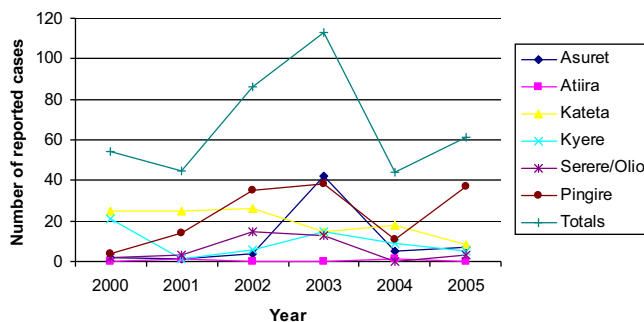


Fig. 9. Sub-counties in Soroti district affected by *T. b. rhodesiense*HAT January 2000–July 2005.

Table 4
Reported *T. b. rhodesiense*HAT (rHAT) cases in 14 parishes hosting intervention sites in Soroti district. Relative change is shown as ↓ for a decrease and ↑ for an increase in the number of reported cases.

Sub-county	Parish	Intervention sites	No. rHAT cases pre-intervention (Jan 00 to Dec 02)	No. rHAT cases post-intervention (Jan 03–July 05)	Change
Bugondo	Kangeta	1	0	0	–
Serere	Okulonyo	1	0	1	↑
	Osuguro	1	14	13	↓
Pingire	Oburin	1	0	2	↑
	Aaropoo	1	0	0	–
	Labori	3	0	0	–
	Pingire	4	1	43	↑
Kateta	Kadapakol	3	1	1	–
	Kateta	1	51	22	↓
	Ojetenyang	1	4	4	–
	Kamusala	3	7	4	↓
Kyere	Kelim	1	9	6	↓
	Kyere	1	8	4	↓
Asuret	Mukura	1	5	25	↑
Atiira	Atiira	2	0	0	–
Total		25	100	124	↑

**Fig. 10.** *T. b. rhodesiense*HAT (rHAT) cases in affected sub-counties Soroti district (January 2000–July 2005).

A single primary cluster of rHAT cases 17.52 km radius, across Kateta and Kyere sub-counties was evident in 2000 (Fig. 11). A significant cluster further west between Kateta and Pingire sub-counties was observed in 2001 and 2002. In 2003 a secondary cluster was located in this area, with the primary cluster moving northeast to Asuret sub-county. In 2004 the primary cluster returned to Kateta and Kyere sub-counties before migrating westwards to the Kateta/Pingire border in 2005. A primary cluster of cases was centered on the Kateta/Pingire border whilst a secondary cluster was located to the north-east in either Kyere (pre-intervention) or neighbouring Asuret (post-intervention) sub-counties (Fig. 12).

4. Discussion

This study reports the first analysis, at district level, of the impact of mass trypanocidal chemoprophylaxis on cattle on the prevalence of *T. b. rhodesiense* parasites in livestock and on the incidence of rHAT. While the impact of the intervention was notably different in the two districts in this study, a single mass trypanocidal intervention in the absence of vector control, can have a lasting impact on the burden of *T. b. rhodesiense* in both cattle and human populations with important implications for control of zoonotic rHAT.

In Kamuli district, endemic for rHAT, the interventions showed a significant and lasting impact on the prevalence of human infective *T. b. rhodesiense* in cattle. Three months after drug administration the prevalence of *T. brucei* s.l. was 3.11%, half that pre-intervention. Twelve-months after intervention the prevalence of *T. brucei* s.l. (4.7%) remained lower than before drug administration (6.75%), indicating long term impact for AAT and HAT in areas, endemic for

rHAT with low tsetse challenge (Mugenyi et al., 2015). Most notably, was the impact of the intervention on the dynamics between non-human infective and human infective subspecies of *T. brucei* s.l. Before intervention a very high proportion, 56%, of *T. b. brucei* s.l. parasites circulating in cattle were identified as human infective by the presence of the SRA gene (Welburn et al., 2001a,b). A single mass treatment of domestic cattle with trypanocidal drug resulted in elimination of *T. b. rhodesiense* across the intervention area. At both three and 12 months after treatment no human infective *T. b. rhodesiense* parasites were identified. While cattle gradually became re-infected with *T. brucei* s.l. the human infective subspecies, *T. b. rhodesiense* failed to re-establish within the *T. b. brucei* s.l. population – the population structure was profoundly altered. This supports there being a fitness cost for trypanosome parasite being human infective when not in a human host (Milligan et al., 1995; Coleman and Welburn, 2004; Welburn et al., 1995, 2008), which suggests that targeting treatments in cattle is ‘pushing’ at an open door to eliminate *T. b. rhodesiense*.

Soroti district, which had, at the time of intervention, only recently experienced its first rHAT outbreak, showed high a prevalence of *T. b. brucei* s.l. in cattle of 21%, three times higher than in Kamuli. The prevalence of *T. b. rhodesiense* prior to intervention was 6%, twice that observed in cattle in Kamuli District. One year post-intervention the prevalence of *T. brucei* s.l. and *T. b. rhodesiense* was half that observed pre-intervention.

Before intervention, 28% of all circulating *T. brucei* s.l. were human infective *T. b. rhodesiense*. There was a significant reduction in the proportion of *T. brucei* s.l. that were human infective after only a single chemotherapeutic intervention and three months after intervention, the proportion of *T. brucei* s.l. identified as *T. b. rhodesiense* was reduced to 10% or 1:10. After 12 months 32% of the *T. brucei* s.l. were again identified as human infective *T. b. rhodesiense*. This re-establishment of the relationship in the population between human infective and non-human infective forms of *T. b. brucei* one-year after intervention was observed despite a reduction in prevalence of *T. brucei* s.l. three months post-intervention. Re-establishment of a 1:3 ratio of *T. b. rhodesiense* within *T. brucei* s.l. samples one-year post-intervention was only observed in Soroti, where a rHAT epidemic was ongoing and may be a function of availability of susceptible animals.

The approximate 1:3 ratio of human infective *T. b. rhodesiense* to non-human infective *T. b. brucei* has been previously reported in non-human hosts (tsetse, domestic and wild animals) in natural systems (Coleman and Welburn, 2004). At the start of the epidemic in Soroti district, 45% of the sampled cattle (n=200) tested posi-

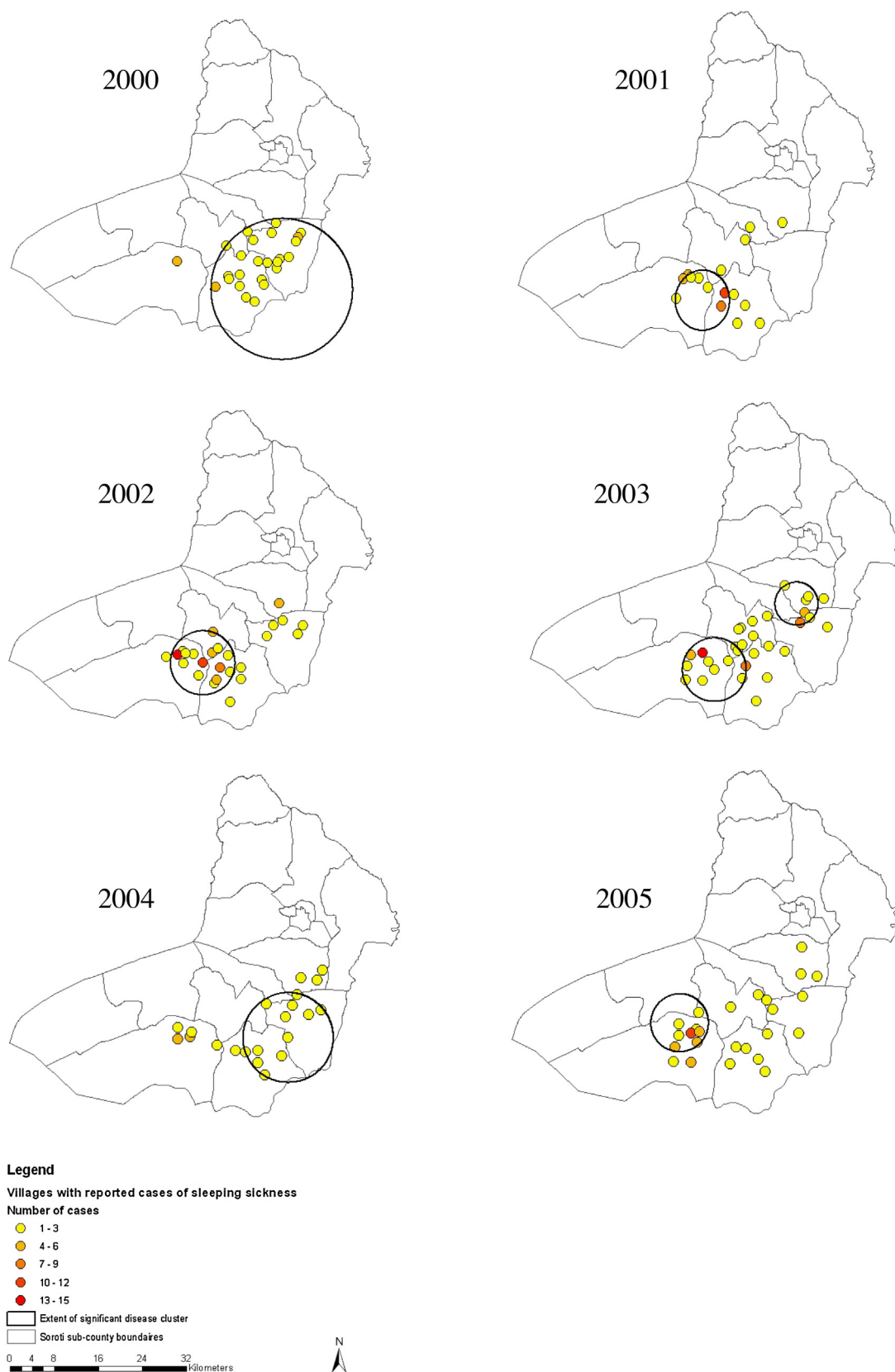


Fig. 11. *T. b. rhodesiense* HAT (rHAT) case distribution in Soroti district and significant rHAT disease clusters (circled) (2000–2005).

tive for *T. brucei* s.l. and 40% of these were characterised as *T. b. rhodesiense* (Welburn et al., 2001b).

Establishing the extent of the reservoir of human infective *T. b. rhodesiense* parasites in cattle not only provides an indicator of risk to the human population in the affected districts but also per-

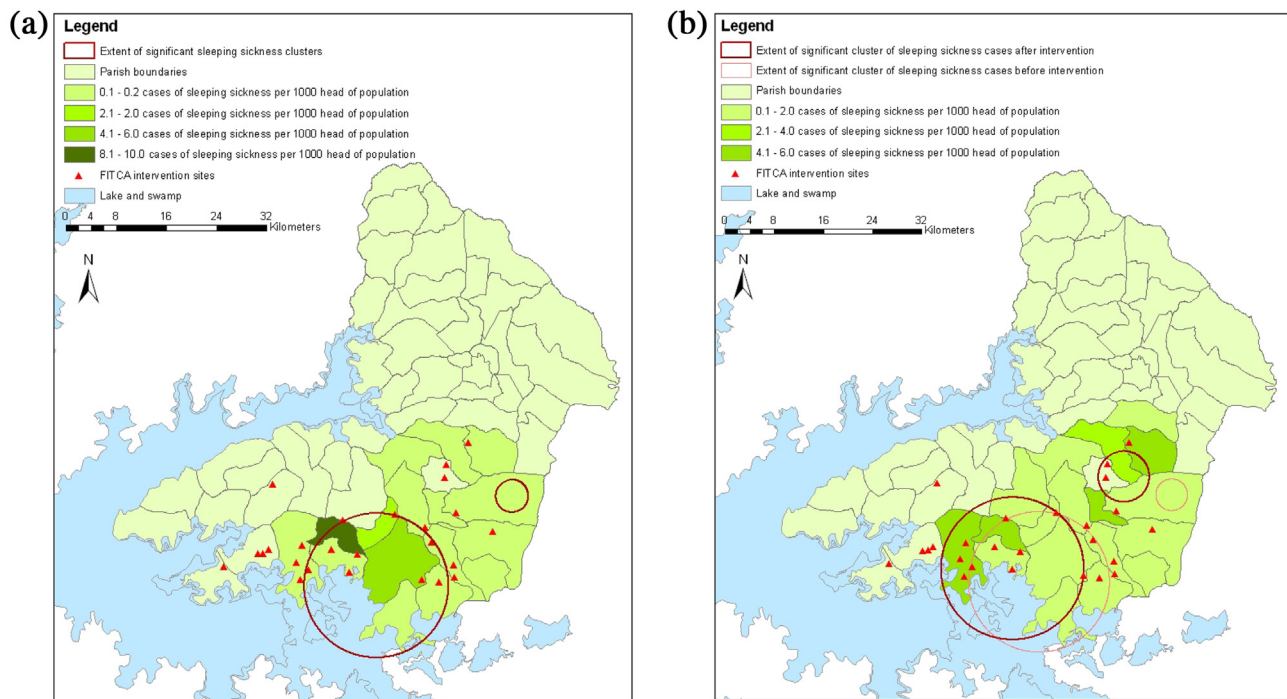


Fig. 12. Annual incidence of *T. b. rhodesiense* HAT cases per parish, Soroti district (a) pre-intervention (January 2000–December 2002) and (b) post-intervention (January 2003–July 2005).

mits appraisal of the risk of the disease spreading to neighbouring districts. The presence of *T. b. rhodesiense* or elevated prevalence of *T. brucei* s.l. in village cattle can be used as a simple indicator of sleeping sickness risk (von Wissmann et al., 2014). The large impact of chemotherapeutic intervention on the proportion of human infective parasites within circulating *T. b. brucei* in cattle offers an opportunity to capitalise on the fitness cost of being human infective when not in a human host for this zoonotic parasite (Coleman and Welburn, 2004).

In Kamuli district, a positive relationship was observed between the impact of the mass treatment in cattle and a decrease in human rHAT cases. The number of rHAT cases reported in Kamuli increased between 2000–2001, before trypanocidal mass intervention in cattle, and decreased in 2002–2005, after intervention. Clustering of rHAT cases was observed in Kamuli, Namwendwa sub-county reported 78% of all rHAT cases with significant case clustering in two parishes in the north-east of the sub-county (Keeya and Ndalike). Clustering of rHAT cases is not an unusual feature of rHAT epidemiology (Zoller et al., 2008). In the nine parishes that hosted intervention sites in Kamuli, the incidence of reported rHAT cases decreased from 5.54 cases per 1,000 head of population in the pre-intervention period to 0.76 cases per 1,000 head of population post intervention. There was no evidence of seasonal variation for rHAT case reporting in Kamuli, indicative of persistent but low tsetse challenge.

In Soroti district, the intervention had minimal impact on the number of rHAT cases reported, with similar annual incidence of reported rHAT cases pre- ($n=218$) and post-intervention ($n=185$). Seasonal variation in rHAT case reporting was observed in Soroti and there was a significant reduction in the seasonal peak of cases in Soroti in 2004. An impact on seasonal case peak in 2003 would not be expected due to a) late reporting of cases and b) the 4- 6-week time delay for *T. brucei* s.l. maturation of infections in tsetse to impact on transmission (Welburn et al., 1995; Milligan et al., 1995). The intervention had negligible impact on the geographical distribution of cases, with significant clusters of reported disease

being similarly located in both pre- and post-intervention periods. At the local level, parishes hosting intervention sites showed an increase in incidence of reported cases post-intervention ($n=127$) but the intervention itself may have promoted awareness of this new disease in the district. There is substantial under-reporting of the disease partially due to low knowledge about symptoms and treatment pathways (Odiit et al., 2005; Acup et al., 2016).

The different epidemiological patterns of case reporting and impact of the interventions in Soroti and Kamuli may be a reflection of the different stages of endemicity of rHAT in these districts. In Kamuli, rHAT has been endemic for a century (Koerner et al., 1995) while rHAT was only introduced into Soroti in 1998. In Kamuli, the large number of cases from a small area, complemented by sporadic reporting of low numbers of cases from other locations, is indicative of recrudescence (and subsequent decline) of rHAT within the Busoga endemic focus. The continuous reporting of high/moderate numbers of rHAT cases across a geographically defined area in Soroti, showing a high degree of motility in the focus of clustered cases, consistent with an ongoing unstable epidemic.

Another important observation related to the observed impact is the level of intervention coverage. There are three reasons why coverage may have been higher in Kamuli. Firstly, the intervention in Kamuli focussed on a single sub-county (Namwendwa) rather than across seven as in Soroti, which would have allowed for increased community sensitisation. Secondly, the DVO and intervention team in Kamuli district were highly experienced in implementing community livestock interventions, that likely influenced community perceptions and confidence. Lastly, Soroti district was a late addition to the wider FITCA development programme, due to the unexpected introduction of rHAT into the district and had not benefited from early engagement (surveys for AAT prevalence and cattle census). Greater intervention success in Kamuli may have also been due to high intervention coverage, of 81% of cattle in Namwendwa sub-county. Concentration of effort in a single sub-county may have been advantageous for sensitisation of the population and operational efficiency. In Soroti, the percentage of the cattle population

treated was low – approximately 40% – substantially below the 86% target articulated by the mathematical model in Coleman and Welburn (2004).

The results show that a single mass trypanocidal intervention targeted solely at the principal reservoir of *T. b. rhodesiense* infection in the absence of tsetse control operations can impact on the burden on *T. b. rhodesiense* rHAT. Evidence from Kamuli shows that a single trypanocidal intervention, applied at scale in an endemic focus can eliminate *T. b. rhodesiense* from the *T. b. brucei* s.l. population of circulating parasites. These gains can be consolidated by additionally implementing preventative measures to stop re-infection from exposure to infected tsetse in the wider environment. The application of trypanocides to remove infection of rHAT and AAT parasites, combined with application of veterinary insecticides to prevent re-infection (Kajunguri et al., 2014; Muhanguzi et al., 2014a) offer an economical control option, with additional benefits for control of tick-borne diseases and AAT (Shaw et al., 2013; Welburn and Coleman, 2015; Muhanguzi et al., 2014b, 2015; Okello et al., 2015).

There is an urgent need to prevent rHAT spreading further towards the gHAT focus. If the foci for rHAT and gHAT merge, diagnosis and treatment protocols will be compromised incurring large public health costs (Welburn and Coleman, 2015). Preventing overlap of gHAT and rHAT will require removal of the reservoir of infection in domestic cattle in Uganda. It is essential to reinforce policy to ensure that animals sold at livestock markets with intent to be moved to a new district, are indeed, given an application of trypanocidal drug to remove the risk of introduction of *T. b. rhodesiense* to new areas (Wendo, 2002; Okello and Welburn, 2014).

The Stamp out Sleeping Sickness (SOS) campaign attempted to scale the FITCA approach (<http://www.stampoutsleepingsickness.com/>) implementing mass cattle treatments (~500,000 cattle treated) in seven districts, north of Lake Kyoga (including Soroti district) between 2006 and 2010. Again, a single trypanocidal drug intervention was implemented, but the SOS intervention included the addition of a pyrethroid based spray application to prevent re-infection and build sustainability. Private veterinarians were established to sell and promote pyrethroid-based insecticides for application to cattle for tsetse control turning livestock into 'live baits' to kill tsetse (Torr et al., 2007; Kajunguri et al., 2014; Bardosh et al., 2013). SOS halted the northwards spread of rHAT cases and reduced the prevalence of all trypanosomes in cattle by 75% across seven districts. Overall, there was a significant decrease in *T. brucei*.l. prevalence in cattle by 67.1%. The prevalence of zoonotic *T. b. rhodesiense* was reduced by 85.7%; from 0.75% to 0.11% with only a single round of mass treatment and two applications of pyrethroid insecticide (Welburn and Coleman, 2015).

The WHO aims to eliminate sleeping sickness in Africa by 2030 (Simarro et al., 2015). With the threat of overlap between *T. b. rhodesiense* and *T. b. gambiense* foci remaining in Uganda, there are plans to implement intensive mass cattle interventions (trypanocides and pyrethroid-based insecticides) to 2.7 million animals in at-risk districts of Uganda. The major hurdle as with all neglected zoonotic diseases is financing, scale-up demands significant upfront investment and long term implementation. An innovative funding mechanism has been proposed to support this large-scale, long-term approach to rHAT control in Uganda – a Development Impact Bond (DIB). Development Impact Bonds (DIB), use “private investment to provide upfront risk capital for development programmes, only calling on donor funding to repay capital, plus a potential return, once clearly defined and measured development outcomes are achieved” (Centre for International Development and Social Finance, 2013). This approach that can harness the appetite of private sector investors for creating social impact as well as deliver financial returns based on performance, bring greater rigour to delivery of international development and global health inter-

ventions – investors will only back strategies that have evidence of success (Welburn et al., 2016b).

Author contributions

JF, KLB and SCW prepared and finalized the paper. JF, SCW, CW, KP contributed in the study conception, design, acquisition of data. JF, SCW, KP and KLB contributed to data analysis and interpretation. JF and SCW wrote the manuscript. All authors read and approved the final manuscript.

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