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The pathway to sustainable elimination of Human African Trypanosomiasis in Democratic Republic of Congo

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To be defended by

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Dedication

In memory of my beloved **Father**, Victor Mbuyi wa Lumbala, my Brother and my first friend, Paul Mbuyi wa Mbuyi and my Sister, my confident, Thérèse Tshisabi wa Mbuyi

To Dr. Miaka Mia Bilenge Constantin†

To Prof. Dr. Marleen Boolaert†

To my loving mother, Marie José Ndaya wa Kazadi

To the Lumbala family: Ms. Liliane Njilabu, Bonheur Mbuyi, Gracia Ngalula, Naomie Mulanga, Benidi Mbuyi, Marie Consolatrice Kasanga, Marie Goretti Ndaya, Yaelle Marie Christie Mujinga

To all the members, children and grandchildren of the great "Wa Mbuyi" family

To any person with a scientific mind, driven by the search for truth, goodness and devoid of any spirit of sectarianism, obscurantism and personal profit, person of good faith, good will.

To the DRC PNLTHA (DRC sleeping Sickness Control Programme)

Declaration

I, Crispin Lumbala wa Mbuyi, declare that this Thesis is my original work. It is being submitted for the degree of Doctor of Medical Sciences at the University of Antwerp, Belgium. It has not been submitted before for any degree or examination at this or any other University, and all the sources used or quoted have been acknowledged by references.

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List of acronyms and abbreviations

AAT: African Animal Trypanosomiasis

ADR: Annual Detection Rate

AO: Acridine Orange

AS: Active Screening

BBB: blood-brain barrier

BC: Buffy Coat

BCT: Bureau Central de la Trypanosomiase

CAR: Central African Republic

CATT-P: CATT on plasma (or serum) dilutions

CATT-WB: CATT on whole blood

CATT: Card Agglutination Test for Trypanosomiasis

CNS: Central Nervous System

CSF: Cerebrospinal fluid

CTC: Capillary Tube Centrifugation technique

DFMO: Difluoromethylornithine

DGLM: Direction Générale de Lutte contre la Maladie

DHSP: Direction de l'Hygiène et Salubrité Publique

DLS: Direction des Laboratoires de la Santé

DNA: Desoxyribonucleic acid

DNDi: Drugs for Neglected Diseases initiative

DOT: Directly observed treatment

DRC: Democratic Republic of the Congo

DSE: Direction de Surveillance épidémiologique

EDTA: ethylene diamine tetra-acetic acid

EHA: endemic Health Area

EHD: endemic Health District

ELISA: Enzyme-linked immunosorbent assays

EoT: elimination of transmission

ES: encephalopathic syndrome

FAO: the Food and Agriculture Organization of the United Nations

gHAT: *Gambiense* HAT

HA: Health Area

HAT: Human African Trypanosomiasis

HD: Health District

HIV: Human Immunodeficiency virus

IFAT: Indirect fluorescent antibody test

IV: intravenous

Kg: Kilogram

LAMP: loop-mediated isothermal amplification

LED: light-emitting diodes

LP: Lumbar Puncture

mAECT-BC: mAECT on buffy coat

mAECT-WB: mAECT on Whole Blood

mAECT: Mini-anion-exchange centrifugation technique

mg: milligram

mHCT: micro-haematocrit centrifugation technique

MSC: modified single centrifugation

MSF: Médecins Sans Frontières

MT: Mobile Team

NASBA: Nucleic acid sequence-based amplification

NECT: nifurtimox - eflornithine combination therapy

NGO: Non-governmental Organization

NSSCP: National Sleeping Sickness Control Program

NTD: neglected tropical diseases

PAAT: the programme against African trypanosomosis

PCR: Polymerase chain reaction

PHC: primary health-care

PHP: public health problem

PNLTHA: Programme National de Lutte contre la Trypanosomiase Humaine Africaine

PS: passive screening

QBC: quantitative buffy coat test

RBC: red blood cell

RDT: rapid diagnostic tests

rHAT: *rhodesiense* HAT

RNA: Ribonucleic acid

T. b: *Trypanosoma brucei*

TL: Trypanolysis test

TSP: Total Screened People

USD: United States Dollar

VSG: variant (or variable) surface glycoprotein

WB: Whole Blood

WBC: white blood cell

WHO: World Health Organization

ZS: Zone de Santé

µl: microliter

Samenvatting thesis

Introductie

Human African Trypanosomiasis (HAT), ook bekend als Slaapziekte, is een door vectoren overgedragen parasitaire ziekte. Het is beperkt tot Afrika ten zuiden van de Sahara, tussen breedtegraden 14 ° N en 29 ° ZB, binnen de grenzen van de geografische verspreiding van de tseetseevlieg van het geslacht *Glossina*, die de ziekte overbrengt. *Trypanosoma brucei (T. b.) gambiense*,wordt gevonden in Centraal- en West-Afrika en veroorzaakt 97-98% van alle gevallen afgezien van *T. b. rhodesiense*, gevonden in het oosten en zuidelijk Afrika. Vanwege de gestage daling van het aantal gevallen, werd de eliminatie *van Gambiense* HAT (gHAT) als volksgezondheidsprobleem (PHP) een doel tegen 2020 dat nu is gevolueerd naar eliminatie van transmissie (EoT) tegen 2030. De Democratische Republiek Congo (DRC), het zwaarst getroffen land, lijkt overduidelijk op weg naar de eliminatie van gHAT. Daarom evalueerden we de ziektetrend van de nationale en subnationale niveaus, evenals de kwaliteit en dekking van gHAT-controleactiviteiten tussen 2000 en 2016 in de progressie naar gHAT-eliminatie in de DRC. We hebben enkele belemmeringen voor een permanente eliminatie van gHAT, aangepakt. Deze belemmeringen waren voornamelijk gerelateerd aan diagnostische hulpmiddelen en strategieën ter ondersteuning van een effectieve integratie van gHAT-controle in het systeem voor de eerstelijnsgezondheidszorg (PHC) en verbetering van de dekking van activiteiten.

Doelstellingen

Dit proefschrift had tot doel bij te dragen aan de eliminatie van gHAT als PHP-doelstelling (in 2020) en EoT (in 2030), door beoordeling van geïmplementeerde controlestrategieën en ontwikkeling van innovatieve en nieuwe diagnostische hulpmiddelen en strategieën.

Specifieke doelstellingen:

- Het beoordelen van de implementatie van de huidige controleactiviteiten simultaan met de prevalentie trend van gHAT in de DRC;
- Het evalueren in termen van efficiëntie, effectiviteit en kosteneffectiviteit van nieuwe diagnostische hulpmiddelen;
- Het evalueren van een geïntensiveerde (opgeschaalde) en innovatieve gHAT passieve screening strategie.

Methodologie

Om de epidemiologische trend van gHAT en controlestrategieën te beoordelen die voldoen aan de richtlijnen in de DRC, hebben we gegevens gebruikt die zijn verstrekt door het DRC National Sleeping Sickness Control Program (NSSCP) en gearchiveerd door de wereldgezondheidsorganisatie (WGO) (Atlas of HAT) (beschikbaar vanaf 2000). We analyseerden de ziektetrend van 2000 tot 2016 op landelijk, provinciaal en

gezondheidsdistrictsniveau. We beoordeelden de bereikte resultaten in de richting van gHAT-eliminatie als PHP op landelijk niveau gedurende een periode van 5 jaar vanaf 2012 (toen de doelstelling om gHAT als PHP tegen 2020 te elimineren werd aangenomen) vergeleken met de periode 5 jaar 2000-2004, (gerapporteerd door Franco et al (2020)). We beoordeelden en vergeleken de intensiteit van gHAT-transmissie per gezondheidsdistrict door het jaarlijkse gemiddelde gHAT-aantal gevallen per 10.000 inwoners voor periodes 2000-2004 en 2012-2016.

Om de overeenkomst van de implementatie van actieve screening (AS) met de aanbevolen algoritmen van 2012 tot 2016 te beoordelen, gebruikten we HAT Atlas-gegevens van 2007 tot 2016 en schatten we

- het aandeel dorpen dat voor AS was gekozen met de aanbevolen AS-implementatiealgoritmen die van 2012 tot 2016 jaarlijks door een mobiel team worden bezocht;
- het aandeel dorpen dat niet was gekozen voor AS met de aanbevolen AS-algoritmen, maar wordt bezocht door een mobiel team, en deteams die gHAT-gevallen hebben gemeld van 2012 tot 2016.

Deze studie werd uitgevoerd in 5 provincies, waaronder Maindombe en Kwilu, 2 provincies die van 2000 tot 2016 sterk endemisch bleven, Sud Ubangi en Kongo Central, waar een opmerkelijke daling optrad, en Kasaï Oriental waar de daling minimaal was.

We beoordeelden de passieve screeningsdekking in 2017 op provinciaal en gezondheidsdistrictsniveau, als het aandeel van endemische gezondheidsgebieden (EHA) en endemische gezondheidsdistricten (EHD), die ten minste één gHAT-geval meldden in de periode van 2012 tot 2016, gedekt door ten minste een screeningsgezondheidsfaciliteit voor een gezondheidsgebied, of door een referentiegezondheidscentrum die gHAT-screening uitvoert, controletesten en alle ziektestadia behandeld (globaal pakket) voor het gezondheidsdistrict.

Om de passieve screeningsdekking te beoordelen, hebben we in 2017 de volgende parameters geëvalueerd:

- Het aandeel EHD met ten minste één gezondheidsinstelling die de gHAT-activiteiten met volledig pakket uitvoert;
- Het aandeel van EHA met ten minste één gezondheidsinstelling die gHAT serologische screening uitvoert;
- Het aandeel EHA-populaties binnen 5-8 km van een zorginstelling die gHAT-serologische screeningtests uitvoert.

Om CATT-beperkingen te overkomen en het probleem van de huidige actieve screening van afnemende kosteneffectiviteit aan te pakken, richtte dit onderzoeksproject zich op diagnostische hulpmiddelen en strategieën, waarbij de effectiviteit van verschillende individuele snelle diagnostische tests werd geëvalueerd; de SD BIOLINE HAT RDT® op basis van inheemse trypanosoomantigenen (1^{e} generatie HAT RDT) en de SD BIOLINE 2® HAT RDT op basis van recombinante antigenen (2^{e} generatie HAT RDT) werd vergeleken met CATT, evenals de efficiëntie van nieuwe diagnostische strategieën die deze individuele tests combineren

met andere innovatieve diagnostische hulpmiddelen zoals 12 V microscoop met behulp van LED-lampen en LAMP moleculaire test. We evalueerden ook een prototype van een gecombineerde HAT/malaria RDT in vergelijking met gHAT en malaria individuele RDT's, meer dan de enkelvoudige RDT, vertegenwoordigt deze gecombineerde RDT een gHAT- en malaria-integratiebenadering.

Resultaten

Van 2000 tot 2016 rapporteerden 20 van de 26 provincies in de DRC ten minste één gHAT-geval, en werden beschouwd als gHAT-endemische provincies. Op nationaal niveau daalde het aantal gescreende gevallen met 89,6% van 16.951 naar 1.768, terwijl het totale aantal gescreende mensen werd gehandhaafd of tegelijkertijd werd verhoogd tot een afnemend jaarlijks detectiepercentage (percentage gedetecteerde patiënten onder alle gescreende mensen), wat een echte afname van de omvang (last) van de ziekte aantoont. Analyse op provinciaal niveau onthulde dat de provincies Kwilu en Maindombe in die periode het meest endemisch waren, gevolgd door de provincies Kasai Oriental en Kongo Central. De provincies Nord Ubangi en Sud Ubangi kenden een sterke daling van het aantal gevallen.

Van de 225 EHD's die ten minste één gHAT-geval van 2000 tot 2004 rapporteerden, waren er 113 (50,2%) met een lage of zeer lage transmissie-intensiteit (<1 gHAT/10.000 inwoners), wat betekent dat de eliminatie van gHAT als PHP-drempel al was bereikt, terwijl 78 op matige transmissie-intensiteit waren (≥1 gHAT/10.000 inwoners & <1gHAT / 1.000 inwoners) en 34 bij hoge of zeer hoge transmissie-intensiteit (≥1 gHAT/1.000 inwoners). In deze periode van 2012 tot 2016 was gHAT nog steeds een PHP in 41 EHD's (18,2%) met 39 met matige transmissie-intensiteit en 2 met hoge of zeer hoge transmissie-intensiteit, wat een afname was met twee derde, vergeleken met de periode 2000-2004. Drie EHD's met een lage of zeer lage transmissie-intensiteit in de periode 2000-2004 stijgden naar een matige intensiteit in de periode 2012-2016.

Na een actieve screening bleek dat de naleving van aanbevolen algoritmen laag was. In de vijf geselecteerde provincies (Kwilu, Maindombe, Kasai Oriental, Kongo Central en Sud Ubangi) werd, van de in aanmerking komende dorpen, 20,0% jaarlijks, 42,6% ten minste tweemaal en 63,7% ten minste één keer onderworpen aan AS gedurende de 3 jaar tussen 2014 en 2016. Met betrekking tot passieve screeningsdekkingsbeoordeling werd 92,7% van de EHD's waarbij gHAT nog steeds een PHP was voor de periode 2012-2016, gedekt door ten minste één referentieinstelling die het volledige pakket gHAT-activiteiten uitvoerde, terwijl slechts 34,4% van de EHD's bij lage en zeer lage transmissie-intensiteit werd gedekt door een volledig pakket gHAT-activiteiten. Bij EHD's bij transmissie met lage en zeer lage intensiteit had 54,6% geen enkele gezondheidsfaciliteit (HF) die serologische screening uitvoerde.

Een prototype snelle diagnostische test voor HAT, de SD BIOLINE HAT RDT®, geëvalueerd door actieve en passieve screening in Angola, de Democratische Republiek Congo en de Centraal-Afrikaanse Republiek, werd net zo gevoelig bevonden als de CATT in een 1/8 verdunning (CATT 1:8) met 89,26% (95%BI=83,27 - 93,28)

gevoeligheid voor elk) en een CATT-WB gevoeligheid van 93,96% (95%BI=88,92 - 96,79) die een grotere steekproefomvang nodig heeft om vergelijkende conclusies te trekken. De totale RDT-specificiteit was inferieur aan CATT-WB en CATT 1:8 met 94,58% (95% BI=94,20 - 94,94), 95,91% (95% BI=95,58 - 96,22) en 98,88% (95% BI=98,70 - 99,04) voor respectievelijk RDT, CATT-WB en CATT 1:8.

Eenmaal geoptimaliseerd bleek de SD BIOLINE HAT RDT® geëvalueerd in DRC gevoeliger te zijn dan CATT-WB en CATT 1:8 mat 92,0% (95%BI=86,1-95,5), 69,1% (95%CI=60,7-76,4) en 59,0% (95%CI=50,2-67,2) voor respectievelijk RDT, CATT-WB en CATT 1:8. De CATT 1:8 had de hoogste specificiteit groter dan CATT-WB en deze laatste superieur aan de RDT met 99,6% (95%BI=99,5-99,7), 98,0%(95%BI:97,8-98,2) en 97,1(95%BI:96,8-97,4) voor respectievelijk CATT 1:8, CATT-WB en RDT. Geconfronteerd met de zeer goede gevoeligheid en zwakke specificiteit van de SD BIOLINE HAT RDT® in vergelijking met CATT was een economische analyse nuttig om de relatieve kosteneffectiviteit van CATT en RDT te meten in drie verschillende diagnostische algoritmen in mobiele teams (actieve screening) en vaste gezondheidsfaciliteiten (passieve screening). In zowel vaste faciliteiten als mobiele teams had screening van deelnemers met behulp van de SD BIOLINE HAT RDT® gevolgd door parasitologische bevestiging een lagere kosteneffectiviteitsratio dan in algoritmen die CATT gebruikten. Algoritmen die de RDT gebruikten, waren goedkoper met respectievelijk 112,54 (33,2%) en 88,54 (32,92%) US dollar per geval, gediagnosticeerd in mobiele teams en vaste gezondheidsfaciliteiten, in vergelijking met algoritmen die CATT gebruiken. Gevoeligheidsanalyse toonde aan dat deze conclusies robuust waren voor een aantal aannames en dat de resultaten kunnen worden geschaald naar kleinere of grotere faciliteiten en een variabele endemiciteit.

Helaas werd de SD BIOLINE HAT RDT® gemaakt van inheemse antigenen, geconfronteerd met de inheemse antigenenproductie-problemen, omdat het afhankelijk is van een arbeidsintensief, kostbaar en riskant proces waarbij ratten worden ingeënt met mens-infectieuze trypanosomen. Om deze uitdaging aan te gaan en de standaardisatie en kwaliteit van de productie te verbeteren, werd een nieuwe RDT geproduceerd met behulp van recombinante antigenen, de SD HAT BIOLINE 2.0®, ontwikkeld en meer gevoelig bevonden in vergelijking dan de SD BIOLINE HAT® en voor vergelijking met CATT was een grotere steekproefomvang nodig heeft om vergelijkende conclusies te trekken met 71,2% (95%BI:65,7-76,6) 59,0% (95%BI:53,0-64,6) en 62,5% (95%BI56,2%-68,4) gevoeligheid van respectievelijk SD BIOLINE HAT 2.0, SD BIOLINE HAT en CATT. De SD HAT BIOLINE 2.0® was minder specifiek dan CATT en SD BIOLINE HAT met 99,2% (95%BI:99,1-99,2), 98,9% (95%BI:98,8-99,0) en 98,1% (95%BI:98,0-98,2) respectievelijk voor CATT, SD BIOLINE HAT en SD HAT BIOLINE 2.0. De gevoeligheid van de tests was lager dan eerder gemeld, omdat ze gevallen identificeerden van gedeeltelijk overlappende subpopulaties. Alle drie de tests waren gevoeliger bij passieve dan bij actieve screening. Het combineren van twee of drie tests resulteerde in een duidelijk

verhoogde gevoeligheid, 99,6% (95%BI: 98,7-100,0) voor alle drie de tests en 90,1% (95%BI: 86,2%-93,6%) voor SD BIOLINE HAT® in combinatie met SD BIOLINE HAT 2.0®.

Actieve screening en zelfs passieve screening zoals momenteel geïmplementeerd, worden minder efficiënt met afnemende incidentie, wat innovatieve strategieën rechtvaardigt om de resterende gevallen efficiënt te detecteren. Daarom hebben we een malaria/HAT gecombineerd prototype RDT geëvalueerd, met het oog op de integratie van gHAT-screening en malariadiagnose. In feite overlappen malaria en gHAT elkaar geografisch en presenteren ze een vergelijkbaar klinisch beeld in het vroege stadium van HAT. Het HAT/Malaria Combined prototype RDT werd net zo nauwkeurig gevonden als de individuele malaria of gHAT RDT's. Voor malaria, met behulp van verse volbloedmonsters, waren de gevoeligheid en specificiteit van de malariaband in de HAT / Malaria Combined RDT respectievelijk 96,9% (95%BI:95,0-98,3) en 97,1% (95%BI:94,1-98,8). De sensitiviteit en specificiteit van de SD BIOLINE malaria Ag P.f. RDT waren respectievelijk 97,3% (95%BI:95,5-98,6) en 97,1% (95%BI:94,1-98,8). Voor gHAT, met behulp van verse volbloedstalen, was de specificiteit van de HAT / Malaria Combined RDT voor gHAT 95,8% (95% BI: 94,3-97,0). Met behulp van gearchiveerde plasmastalen (vanwege het feit dat bij een zeer lage prevalentie verse bloedsta geen nauwkeurige evaluatie van de gevoeligheid mogelijk maakten), waren de sensitiviteit en specificiteit respectievelijk 89% (95%BI:84,4-92,6) en 93,5% (95%BI:89,7-96,2) met de HAT / Malaria Combined RDT, en 88,2% (95%BI:83,5-92) en 94,7% (95%BI:91.1-97.2) met de HAT 2.0 RDT®

Op basis van al deze ontwikkelde RDT's en andere innovatieve diagnostische nieuwe hulpmiddelen, waaronder LAMP, een moleculaire test, en 12V LED-microscoop, werd in de provincie ‘Kongo Central’ van de DRC, een geïsoleerde provincie die een relatief laag, maar stabiel aantal gevallen rapporteert, een strategie om de passieve screeningdekking en vroege detectie van HAT-gevallen uitgerold. In deze strategie werden bijna alle gezondheidsfaciliteiten in de provincie uitgerust met RDT's om patiënten te testen die HAT gerelateerde symptomen vertoonden, werd één gezondheidsfaciliteit per gezondheidsdistrict geüpgraded om microscopie bevestigende tests uit te voeren, en een beperkt aantal om LAMP-test uit te voeren op stalen van onbevestigde RDT+ ve-gevallen. Deze strategie bleek succesvol te zijn in vergelijking met de vorige. Deze strategie verkleinde de afstand die een patiënt aflegt naar een faciliteit die screent op HAT, van 13,7 km naar 3,4 km. Van augustus 2015 tot december 2016 steeg het aandeel van de gedetecteerde HAT-gevallen, jaarlijks gecorrigeerd, met 30% tot 130% in vergelijking met de voorgaande twee jaar, en 64,2% van hen was in een vroeg stadium van de ziekte, vergeleken met 28,3% en 26,7% eerder in 2013 en 2014. Deze strategie heeft een betere bevolkingsdekking mogelijk gemaakt, en wanneer aangevuld met reactieve screening (waarbij actieve screening is gericht op dorpen die passief en in hun buurt gevallen hebben gemeld), maakte het de identificatie van lokale uitbraken en vroege detectie van de meeste gevallen mogelijk, wat van cruciaal belang is bij het

verwijderen van het HAT-reservoir en het onderbreken van de overdracht, waardoor wordt bijgedragen aan de eliminatie van de ziekte.

Discussie

Op basis van gegevens van 2012 tot 2016 had 81,8% van de HD's die tussen 2000 en 2016 gHAT-gevallen meldden, een lage of zeer lage transmissie-intensiteit en bereikten ze de drempel voor eliminatie van gHAT als PHP; waaruit blijkt dat DRC al op weg was naar gHAT-eliminatie. Helaas voldeden de activiteiten die aan deze prestatie ten grondslag lagen in slechte mate aan de algoritmen en richtlijnen en lijken ze niet voldoende te zijn om toezicht te houden in de periode na eliminatie en voldoende vertrouwen te geven in duurzame evolutie naar een EoT. Het feit dat er tussen 2020 en 2021 een relatieve toename van het aantal gevallen in het land werd vastgesteld, versterkt het besef om waakzaam te blijven. Deze resultaten moeten uiteindelijk verder worden onderzocht; of de grote afname van de HAT ziektelast is een werkelijkheid, of er is een verborgen explosieve situatie, wat zeer onwaarschijnlijk is, omdat er geen uitbraak of geruchten van een uitbraak zijn gehoord.

De echte uitdaging is om een herintroductie van gHAT te voorkomen, vooral wanneer de dekking niet voldoende is en als de actieve screening niet wordt gehandhaafd op regelmatige basis. Daarom zal het noodzakelijk zijn om te zorgen voor duurzame en effectieve bewaking van gHAT in foci waar de ziekte is geëlimineerd als PHP en om de dekking van HAT activiteiten te verbeteren. Om dit probleem aan te pakken, hebben we ons onderzoek gericht op innovatieve diagnostische hulpmiddelen, met name de evaluatie van RDT's in vergelijking met CATT's en de ontwikkeling van innovatieve strategieën op basis van deze RDT's en andere innovatieve tools om de integratie van activiteiten in het PHC-systeem te vergemakkelijken.

De eenvoud en stabiliteit van de HAT RDT heeft een geweldige kans gecreëerd om de screeningsdekking van de risicopopulatie te verbeteren, omdat deze kan worden ingezet in elke gezondheidsfaciliteit in endemische gebieden. Door CATT-zwakke punten op te lossen, vergemakkelijkt HAT RDT de integratie van HAT-screening in PHC-instellingen en dus de dekking van passieve screening aangevuld met reactieve screening wanneer en waar nodig. Ons onderzoeksproject heeft bijgedragen aan de integratie van HAT-diagnostiek met andere tropische ziekten zoals malaria, cruciaal nu HATe minder vaak voorkomt.

Verdere evaluaties zijn nodig met betrekking tot HAT RDT I om de problemen op te lossen met betrekking tot de specificiteit, haalbaarheid en kosteneffectiviteit in verschillende omstandigheden, waaronder gebruik van verschillende RDT-associaties en diagnostische algoritmen Verder onderzoek in veldomstandigheden is belangrijk met betrekking tot de HAT/malaria gecombineerde RDTs. Strategieën en algoritmen om opvolging in de periode na eliminatie te garanderen, moeten worden gedocumenteerd in de richtlijnen van het land op basis van deze nieuwe hulpmiddelen en strategieën.

Summary of the thesis

Introduction

Human African Trypanosomiasis (HAT) also known as Sleeping Sickness is a vector-borne parasitic disease caused by *Trypanosoma brucei* (*T. b.*) *gambiense*, found in Central and Western Africa, which causes 97-98% of all cases and by *T. b. rhodesiense*, which is found in the Eastern and Southern Africa. Human African Trypanosomiasis is limited to sub-Saharan Africa, between latitudes 14°N and 29°S, within the limits of the geographical distribution of the tsetse fly of *Glossina* genus, that transmits the disease. Due to steady decreasing in cases, *Gambiense* HAT (gHAT) was originally targeted for elimination as public health problem (PHP) by 2020; it is now targeted for elimination of transmission (EoT) by 2030.

The Democratic Republic of the Congo (DRC) which is the most affected country, seems inexorably moving towards the elimination of gHAT. Therefore, we evaluated the disease trend from the national and subnational levels as well as the quality and coverage of gHAT control activities between 2000 and 2016 towards gHAT elimination in DRC. We addressed some challenges identified for a sustained elimination of gHAT mainly related to diagnostic tools and strategies to support an effective integration of gHAT control into Primary Health Care (PHC) system and activities coverage improvement.

Objectives

This thesis aimed to contribute to the elimination of gHAT as PHP objective (in 2020) and EoT (in 2030), through assessment of control strategies implemented and development of innovative and new diagnostic tools and strategies.

Specific objectives:

- To assess the implementation of current control activities alongside the prevalence trend of gHAT in DRC;
- To evaluate in term of efficiency, effectiveness and cost-effectiveness new diagnostic tools;
- To evaluate an intensified (scaled up) and innovative gHAT passive screening strategy.

Methodology

To assess the epidemiological trend of gHAT and control strategies compliance to guidelines in the DRC, we used data provided by DRC National Sleeping Sickness Control Program (NSSCP) and archived by WHO (Atlas of HAT) (available from 2000). We analysed the disease trend from 2000 to 2016 at country, provincial and health district levels in view of the gHAT elimination goal. We assessed the achievement towards gHAT elimination as PHP at country level during 5-year period from 2012 (when the objective to eliminate gHAT as PHP by 2020 was adopted) compared to 2000-2004 5-year period, according to Franco *et al* (2020). We assessed and compared the intensity of gHAT transmission per health district through the yearly average gHAT case number among 10,000 inhabitants for 2000-2004 and 2012-2016 periods.

To assess the compliance of active screening (AS) implementation with the recommended algorithms during 5-year period from 2012 to 2016, we used HAT Atlas data from 2007 to 2016 and estimated:

- the proportion of villages targeted for AS according to the recommended AS implementation algorithms visited by a mobile team yearly from 2012 to 2016;
- the proportion of villages not targeted for AS according to recommended AS algorithms, but visited by a mobile team, and those among them that reported gHAT cases from 2012 to 2016.

This assessment was carried out in five provinces including Maindombe and Kwilu, two provinces that remained highly affected from 2000 to 2016, Sud Ubangi and Kongo Central, where a remarkable decrease occurred, and Kasaï Oriental where the decrease was minimal.

We assessed the passive screening (PS) coverage in 2017 at provincial and health district levels, as the proportion of endemic health districts (EHD) and endemic health areas (EHA) that reported at least one gHAT case from 2012 to 2016, covered by a referential health facility implementing gHAT screening, confirmatory and all stages case management (full package) for the health district, or by at least a screening health facility, for the health areas. Regarding EHAs coverage we evaluated also the proportion of EHA populations within 5-8 km of a healthcare facility performing gHAT serological screening tests.

To overcome the Card Agglutination Test for Trypanosomiasis (CATT) weakness and address the issue of current active screening decreasing cost-effectiveness, this research project focused on diagnostic tools and strategies, evaluating the effectiveness of different individual rapid diagnostic tests (RDT), the SD BIOLINE HAT RDT® developed based on native trypanosome antigens (1st generation SD BIOLINE HAT RDT) and the SD BIOLINE 2 HAT RDT® developed based on recombinant antigens (2nd generation SD BIOLINE HAT RDT) in comparison to CATT as well as the efficiency of new diagnostic strategies combining these individual tests and other innovative diagnostic tools among others like 12 V microscope using LED lamps and LAMP molecular test. We evaluated too, a prototype of a combined HAT / malaria RDT in comparison to gHAT and malaria individual RDTs, more than a simple RDT, this combined RDT represents a gHAT and malaria integration approach.

Results

From 2000 to 2016, 20 of the 26 provinces in the DRC reported at least one gHAT case, counted as gHAT endemic provinces. At the national level, the number of cases decreased by 89.6% from 16,951 to 1,768, while the total number of people screened was maintained or increased concomitantly to a decreasing annual detection rate (proportion of patients detected among all people screened), demonstrating a real decrease in the extent (burden) of the disease. Analysis at provincial level revealed that Kwilu and Maindombe provinces remained the most affected areas over that time-period, followed by Kasai Oriental and Kongo Central provinces. Nord Ubangi and Sud Ubangi provinces experienced deep decreasing of number of cases.

Among 225 EHDs that reported at least one gHAT case from 2000 to 2004, 113 (50.2%) were at very low or low transmission intensity (<1 gHAT/10,000 inhabitant), meaning that the elimination of gHAT as PHP threshold was already achieved, while 78 were at moderate transmission intensity (≥1 gHAT/10,000 inhabitant & <1 gHAT/1,000 inhabitant) and 34 at high or very high transmission intensity (≥1 gHAT/1,000 inhabitant). Considering the period from 2012 to 2016, gHAT was still PHP in 41 EHDs (18.2%) with 39 at moderate transmission intensity and 2 at high or very high transmission intensity, as a decreasing of two thirds, compared to 2000-2004 period. However, three EHDs with low or very low transmission intensity in 2000 – 2004 period turned at moderate level during 2012-2016 period.

Active screening assessment revealed that compliance with algorithms was low. In the five sampled provinces (Kwilu, Maindombe, Kasai Oriental, Kongo Central and Sud Ubangi), only 20.0% of eligible villages were subjected to AS yearly between 2014 and 2016, 42.6% at least twice and 63.7% at least once over the 3 years. Regarding PS coverage assessment, 92.7% of EHDs with moderate, high, or very high transmission intensity of gHAT transmission, where gHAT was still a PHP for the 2012-2016 period, were covered by at least one referral health facility (HF) implementing the full package of gHAT activities, while only 34.4% of EHDs at low- and very low- transmission intensity were covered by a gHAT full package activities HF, and 54.6% of these EHDs were covered by neither gHAT full package activities HF nor by serological screening HF.

Concerning alternative screening tools in comparison to CATT, a prototype RDT for HAT, the SD BIOLINE HAT RDT® was evaluated through active and passive screening in Angola, DRC and the Central African Republic (CAR), and found as sensitive as the CATT in a 1/8 dilution (CATT 1:8) (89.26% (95%CI=83.27–93.28) of sensitivity for each one) and less sensitive than CATT on whole blood (CATT-WB), with a non-statistically significant difference [with a CATT-WB sensitivity of 93.96% (95%CI=88.92–96.79)] The overall RDT specificity was inferior to CATT-WB and CATT 1:8 with statistically significant differences [94.58% (95%CI=94.20–94.94), 95.91% (95%CI=95.58–96.22) and (98.88% (95%CI=98.70–99.04) for RDT, CATT-WB and CATT 1:8 respectively].

Once optimized the SD BIOLINE HAT RDT® evaluated in DRC, was found to be more sensitive than CATT-WB and CATT 1:8 with statistically significant differences [92.0% (95%CI=86.1–95.5), 69.1% (95%CI=60.7–76.4) and 59.0% (95%CI=50.2–67.2) for RDT, CATT-WB and CATT 1:8 respectively]. The CATT 1:8 had the highest specificity significantly greater than CATT-WB and this latter significantly superior to the RDT [99.6% (95%CI=99.5–99.7), 98.0% (95%CI=97.8–98.2) and 97.1 (95%CI=96.8–97.4) for CATT 1:8, CATT-WB and RDT respectively]. Given the very good sensitivity of the SD BIOLINE HAT RDT® contrasting with a low specificity in comparison to CATT, in order to guide an informed and rational choice, an economic analysis was carried out and allowed to measure the relative cost-effectiveness of CATT and RDT in three different diagnostic algorithms in mobile teams (active screening) and fixed health facilities (passive

screening) as the average cost per case diagnosed at the prices applicable and sensitivities and specificities experienced in the DRC, collected during this study project. In both fixed facilities and mobile teams, screening of participants using the SD BIOLINE HAT RDT® followed by parasitological confirmation had a lower cost-effectiveness ratio than in algorithms using CATT. Algorithms using the RDT were cheaper by 112.54 (33.2%) and 88.54 (32.92%) US dollars per case diagnosed in mobile teams and fixed health facilities respectively, when compared with algorithms using CATT. Sensitivity analysis demonstrated that these conclusions were robust to a number of assumptions, and that the results can be scaled to smaller or larger facilities, and a range of prevalence.

Unfortunately, the SD BIOLINE HAT RDT made of native antigens, was facing the native antigens production challenges, as it relies on a labour-intensive, costly and risky process that involves inoculating rats with human-infective trypanosomes. To address this challenge, and to improve standardization and quality of manufacturing, a new RDT produced using recombinant antigens, the SD HAT BIOLINE 2.0 RDT®, has been developed and found to be the most sensitive compared to CATT (with no statistically significant difference) and to the SD BIOLINE HAT RDT® (with a statistically significant difference) [71.2% (95%CI:65.7-76.6), 59.0% (95%CI:53.0-64.6) and 62.5% (95%CI:56.2-68.4) as sensitivity of SD BIOLINE HAT 2.0 RDT®, CATT and SD BIOLINE HAT RDT® respectively]. The SD HAT BIOLINE 2.0® was less specific than CATT and SD BIOLINE HAT with statistically significant differences [99.2% (95%CI:99.1-99.2), (98.9% (95%CI:98.8-99.0%) and 98.1%(95%CI:98.0-98.2) respectively for CATT, SD BIOLINE HAT RDT® and SD HAT BIOLINE 2.0 RDT®]. Sensitivity of the tests was lower than previously reported, as they identified cases from partially overlapping sub-populations. All three tests were significantly more sensitive in passive than in active screening. Combining two or three tests resulted in a markedly increased sensitivity, 99.6% (95%CI:98.7-100.0) for all three tests and 90.1% (95%CI:86.2-93.6%) for SD BIOLINE HAT RDT® combined with SD BIOLINE HAT 2.0 RDT®.

Active screening and even passive screening as currently implemented become less efficient with declining incidence, justifying innovative strategies to efficiently detect the remaining cases. Therefore, we evaluated a malaria/HAT combined prototype RDT, in view to facilitate integration of gHAT screening and malaria diagnosis. In fact, malaria and gHAT are overlapping geographically and present similar clinical figure during early stage of HAT. The HAT/Malaria Combined prototype RDT was found as accurate as the individual malaria or gHAT RDTs. For malaria, using fresh whole blood samples, the sensitivity and specificity of the malaria band in the HAT/Malaria Combined RDT were 96.9% (95%CI:95.0–98.3) and 97.1% (95%CI: 94.1–98.8) respectively. The sensitivity and specificity of the SD BIOLINE malaria Ag *P.f.* RDT were 97.3% (95%CI:95.5–98.6) and 97.1% (95%CI:94.1–98.8) respectively. For gHAT, using fresh whole blood samples, the specificity of the HAT/Malaria Combined RDT for gHAT was 95.8% (95%CI:94.3–97.0). Using archived

plasma samples (due to the fact that at very low prevalence, fresh blood samples could not allow an accurate evaluation of sensitivity), the sensitivity and specificity were respectively 89% (95%CI:84.4–92.6) and 93.5% (95%CI:89.7–96.2) with the HAT/Malaria Combined RDT, and 88.2% (95%CI:83.5–92) and 94.7% (95%CI:91.1– 97.2) with the SD BIOLINE HAT 2.0 RDT®.

Based on all these developed RDTs and other innovative diagnostic new tools including LAMP, a molecular test, and 12V LED microscope, a strategy was developed to increase passive screening coverage and early detection of HAT cases in Kongo Central province of DRC, an isolated province reporting a relatively low, yet steady number of cases. To implement this strategy, almost all HF in the province were equipped with HAT RDTs to test patients presenting with symptoms suggestive of HAT, one HF per health district was upgraded to perform microscopy confirmatory testing, and a limited number of HF (an average of 1 HF for 6 health district) was upgraded to perform LAMP test on sample collected from unconfirmed RDT+ve suspects. This strategy was found to be successful compared to previously. This strategy reduced the distance of patient travels to a facility screening for HAT, from 13.7 km to 3.4 km. From August 2015 to December 2016, the proportion of HAT cases detected, adjusted annually, increased between 30% and 130% compared to 2023 and 2014, and 64.2% of them were in early-stage disease, compared to 28.3% and 26.7% during the previous two years. This strategy enabled better population coverage, and when supplemented with reactive screening (whereby active screening is targeted at villages that reported cases passively and in their neighborhood), it allowed the identification of local outbreaks and early detection of most cases, which is critical in removing the HAT reservoir and interrupting transmission, thus contributing to elimination of the disease.

Discussion

Based on data from 2012 to 2016, 81.8% of the HDs that reported gHAT cases from 2000 to 2016, were at low or very low transmission intensity, reaching the threshold for elimination of gHAT as PHP; showing that DRC was already moving toward gHAT elimination. Unfortunately, the activities underpinning this achievement were poorly compliant with algorithms and guidelines, and appear not to be sufficient to guarantee surveillance in the post-elimination period and to provide sufficient confidence in sustainability towards an EoT. The fact that a relative increase in the number of cases was noted in the country between 2020 and 2021, reinforces the need to remain vigilant. These results should eventually be investigated further; either the results showing a large decrease in disease burden were really good, or there was a hidden explosive situation, which is very unlikely, as no outbreak or rumored outbreak occurred.

The real challenge is to prevent a resurgence of gHAT especially when the coverage is not sufficient, and if the regularity of active screening is not maintained. Therefore, it will be necessary to ensure sustainable and effective surveillance of gHAT in foci where the disease has been eliminated as PHP and to improve activities coverage. Thus, to address this issue, we focused our research on innovative diagnostic tools, particularly the

evaluation of HAT RDTs in comparison to CATT and the development of innovative strategies based on these RDTs and other innovative tools to facilitate the integration of gHAT activities into the PHC system.

The simplicity and stability of the HAT RDT has created a great opportunity to improve screening coverage of the population at risk, as it can be deployed to any HF in endemic areas. By solving CATT weaknesses, HAT RDT facilitate the integration of HAT screening in PHC settings and thus the coverage of passive screening complemented by reactive screening when and where needed. Our research project contributed to allow integration of HAT diagnostic into other tropical disease like malaria, which is crucial now that the HAT is less prevalent.

Further evaluations are needed regarding HAT RDT in view to solve the issue regarding the specificity, feasibility and cost-effectiveness in different settings including various RDT associations and diagnostic algorithms. Further investigations in field conditions are important regarding the HAT / malaria combined RDT. Strategies and algorithms to ensure surveillance in post-elimination period must be documented in country's guidelines based on these new tools and strategies.

Chapter 1

General introduction

1. Human African Trypanosomiasis

1.1. Background

Human African Trypanosomiasis (HAT) also known as Sleeping Sickness is a vector-borne parasitic disease. It is limited to sub-Saharan Africa, between latitudes 14°N and 29°S, within the limits of the geographical distribution of the tsetse fly of *Glossina* genus, that transmits the disease. The extracellular protozoan parasite belongs to the genus *Trypanosoma*, species *brucei*, that causes the disease. Two subspecies of *Trypanosoma brucei* (*T. b.*) are pathogenic to humans causing 2 different forms of the disease; *T. b. rhodesiense*, localized in the Eastern and Southern Africa and *T. b. gambiense* found in the Central and Western Africa. The Rift Valley represents the classical geographic separation of the two forms of the disease. If HAT is not treated almost all patients will die (1, 2). About 97-98% of all cases are due to *T. b. gambiense* that causes the chronic form of the disease (*gambiense* HAT) while *T. b. rhodesiense* causes the acute form of the disease (3, 4).

The World Health Organization (WHO) currently recognizes 20 diseases and disease groups which disproportionately affect populations living in poverty, causing important morbidity and mortality with a devastating human, social and economic impact, including stigma and discrimination, on these impoverished communities, as neglected tropical diseases (NTDs) including HAT. Ten of these diseases are targeted for either elimination or eradication. *Gambiense* HAT (gHAT) is among them and was targeted for elimination as public health problem (PHP) by 2020 and now it is targeted for elimination of transmission (EoT) by 2030 (4-6).

People are mainly affected by HAT through bite by infected tsetse flies, that inject the parasite from infected person to a healthy one. In addition other less common routes of transmission of HAT have been reported, including vertical transmission (from mother to child) that induces congenital HAT, accidental mechanical transmission in laboratories, infection through blood transfusion and parasite transmission through sexual contact (1, 7-10).

The disease evolves in 2 phases, a 1st or early stage, and a 2nd or late stage. After a variable period, much shorter (few weeks, 3 to 8) in *rhodesiense* HAT (rHAT) than in gHAT (a mean of 300–500 days), the 1st stage, where parasites dwell in the lymphatic system, bloodstream and peripheral organs (hemo–lymphatic stage), is followed by the 2nd stage of the disease that occurs once the parasite crosses the blood-brain barrier (BBB) and invades the cerebrospinal area (meningo–encephalitic or nervous stage). The average duration of gHAT, the chronic form of the disease is almost 3 years where a rHAT patient progresses to death within 6 months (1, 3).

Although it is recognized that untreated, almost all patients suffering from HAT will die; some cases of self-cure have been reported (11).

Human being is the main reservoir for gHAT, an anthroponotic disease, where animals play a minor role as reservoir (1). In fact gHAT control measures targeting the human reservoir have been highly successful in reducing gHAT transmission (3). Aside this potential role that could be played by animals as reservoirs, seropositive non confirmed people with long-lasting serological responses that could suggest trypano-tolerance phenomenon among human as observed in animals has been reported. Those asymptomatic long-lasting seropositive people may constitute human reservoir of parasite, and contribute to maintain the transmission of gHAT (3, 9, 11). Animals (livestock and wildlife) constitute the reservoir for rHAT, which is a zoonosis, where human are accidently infected (1, 3).

In this thesis, we focused only on the gHAT.

1.2. Epidemiology

The epidemiology of the disease results from the interaction between the trypanosome (the parasite), tsetse flies (the vector) with human and animal hosts as well as the environment within which vectors and hosts evolve. Indeed, infection requires association of the three elements of the "epidemiological triangle": human host as a reservoir, and tsetse fly as carrier of the parasite in an appropriate environment (3).

The 31 species and subspecies of tsetse flies are classified, according to their habitat, in 3 groups: *Glossina fusca* (forest), *Glossina morsitans* (savannah) and *Glossina palpalis* (riverine and forest). In natural conditions almost exclusively flies of the palpalis group (especially *G. fuscipes* and *G. palpalis*), found in western and central Africa, are transmitting *T. b. gambiense* while flies of morsitans group (*G. morsitans, G. swynnertoni*, and *G. pallidipes*), mainly located in East Africa, are involved in the transmission of *T. b. rhodesiense* (1, 3).

The risk of contracting gHAT is increased in relation with human activities such as hunting, fetching firewood, farming, fetching water, washing clothes or food (cassava), artisanal extraction of palm oil or cocoa, brewing, gold and diamond mining, fishing... Young adults are the more affected by the disease as they are the most involved in these productive activities. Male are most affected or equally to female according to at-risk activity in the area (1). Occasionally gHAT cases have been diagnosed in non-endemic countries among migrants and travellers who lived in endemic areas (12). Although gHAT is considered as a rural disease, as it occurs mainly in remote rural areas where populations have low-income levels, some cases of gHAT have been reported in urban and suburban settings (1, 13-16).

The first description of Sleeping Sickness was around the 14th century, by the Arab historian Ibn Khaldun. In fact, Sleeping Sickness is known long time before the colonial period with occasional outbreaks. Outbreaks occurred late in 18th and early 19th centuries. Social, ecological, economic equilibrium disruption that occurred

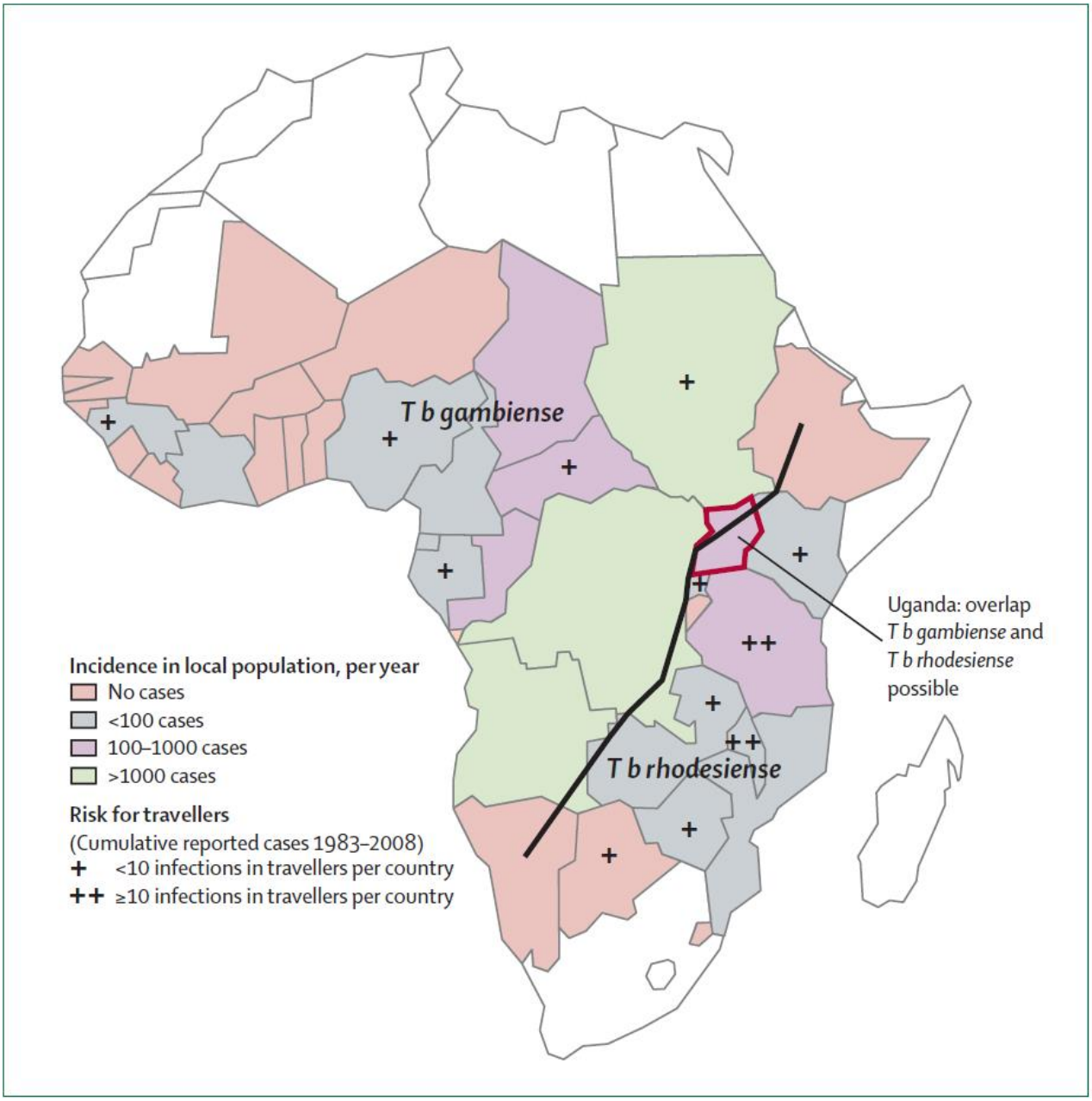
during that period, made humans more in contact with the tsetse flies; and therefore, the causative agent which was identified during the 1st decade of the 20th century (1902, 1903, 1910) (17). Outbreaks of gHAT that occurred at the end of the 19th century and at the beginning of the 20th century (1896–1906), and the one that occurred in the 1920s and 1930s, pushed colonial governments to implement control measures that initially targeted the vector and later the human reservoir. A mobile team was visiting each village at-risk of gHAT to screen the entire population and treat the detected cases. This strategy, conceived and first implemented by Jamot in Ubangi-Shari followed by Cameroon (17), was so successful that the disease was under control at the dawn of independence in early 1960s in most African countries. Following the independence, lack of interest in disease surveillance, decrease in disease control, weakened health system, lack of resources to maintain the active surveillance by mobile teams associated with the conflict and sociopolitical instability in most of gHAT endemic countries (Angola, Democratic Republic of Congo, South Sudan, Central African Republic, Uganda), the disease reemerged in 1970s-1980s, to reach epidemic levels comparable to the one in 1920s (1, 18, 19).

The meeting of the WHO expert committee on the control and surveillance of African Trypanosomiasis held in Geneva, 21-27 November 1995, reported that 36 countries were affected by HAT, 24 by gHAT and 13 by rHAT, Uganda being the only one country where the two forms of HAT coexist (Fig 1). Around 60 million of people were estimated at-risk of sleeping sickness and an average of 300,000 infected new cases annually. Four millions of people at-risk were estimated to be under surveillance and 30,000 among them were detected and treated yearly (2). In 2006, considering the improved knowledge of HAT distribution, WHO reviewed the gap between cases reported and cases estimated to be infected from factor ten to three. Therefore 50,000 to 70,000 were estimated to be infected as 17,500 news cases were reported yearly (18, 20).

The engagement and support of different partners, international organizations, key pharmaceutical companies and international donors under coordination of WHO to endemic countries at the end of 20th century and beginning of the 21st century allowed the reinforcement of gHAT control measures (active and passive case detection, access to treatment, improvement of epidemiological knowledge of the disease). This effort resulted in a substantial progressive decrease of the disease incidence. From the 37,385 and 25,865 gHAT cases reported in 1998 and 2000 respectively, the number of cases decreased to 6,228 cases in 2013, a reduction of at least 80% and 70% compared to 1998 and 2000 respectively. Based on these achievements, the WHO Expert Committee on Control and Surveillance of HAT, the London Declaration on Neglected Tropical Diseases and the 66th World Health Assembly targeted, endorsed and adopted the objective to eliminate gHAT as a public health problem (PHP) by 2020 and to interrupt gHAT transmission by 2030 (1, 3, 21).

The Democratic Republic of the Congo (DRC) remains the most affected country; it accounted for the 70.4% of all gHAT cases in 1998, followed by Angola (17.7%), South Sudan (4.6%), Central African Republic (CAR) (2.9%) and Uganda (2.6%), as much as for the cumulated period from 1998 to 2016 (68.6%, 12.9%, 7.3%,

4.0% and 2.2% for DRC, Angola, South Sudan, CAR and Uganda respectively). In 2016, DRC accounted for 83.8% of all gHAT cases followed by Guinea (5.1%), CAR (4.8%), Chad (2.6%) and Angola (0.9%) (3, 22). South Sudan became an independent State on 9 July 2009, all the cases reported up to that date in former Sudan correspond to the current South Sudan



1.1 Figure 1. Distribution of human African trypanosomiasis with incidences and risk for travellers (23)

1.3. Clinical signs and symptoms, diagnosis and gHAT case management

1.3.1. Clinical signs and symptoms.

The gHAT progresses chronically to late stage and death within 1 to 3 years and shows sometimes latent periods. Symptoms and signs are variable and inconstant (24). Indeed, the clinical signs and symptoms are nonspecific, and their frequency varies between individuals and disease foci. At the beginning, an infected person could remain asymptomatic or pauci-symptomatic for long time while being infectious for other and evolving from 1st to 2nd stage of the disease. The initial sign is a trypanosomal chancre at the site of the infective tsetse bite, however this is a sign that occurs exceptionally. Following signs and symptoms are the most found during the 1st stage of the disease, isolated or diversely associated: general symptoms (including asthenia, general malaise, fatigue, weakness), fever, lymphadenopathies, typically cervical (Winterbottom sign), headache, weight loss, rush, itching (pruritus), anaemia, musculoskeletal pains, hepatosplenomegaly, facial oedema ("puffy-face") and endocrine disturbances. The latter could bring to signs like permanent feeling of cold, myxoedema, dry skin, loss of libido, sexual impotence, amenorrhea, abortion, premature births, sterility. Cardiovascular manifestations are very rare, but electrocardiogram changes are frequent (QTc prolongation, repolarization changes, low voltage). (1, 3, 24).

Neurological signs and symptoms are common to 2nd stage of the disease when the parasite invades the central nervous system (CNS). Partially explained by the predominant location of the brain lesions, they include sleep disturbances, speech disorders, deep sensory disturbance (Kerandel's sign, hyperaesthesia, paraesthesia, anaesthesia), disorders of tone and mobility, abnormal movements, mental and behaviour disorders, psychiatric problems (like anxiety, mood instability, irritability, loss of ability to concentrate, clumsiness, temporospatial disorientation, apathy and negligence in hygiene, reduction of activities, and manic or depressive episodes), convulsion and coma. Sleep disturbances which are characteristic of the disease can also be reported as first signs by the patients or family member; it's characterized by a progressive disruption of the sleep–wake cycle during 24 hours, with daytime somnolence, uncontrollable urge to sleep, and night-time insomnia. However, most of symptoms of the 2 stages overlap, making a stage diagnostic based on the clinical features difficult and unclear so that the final diagnostic is relied on the analysis of the cerebrospinal fluid (CSF) (1, 3, 24, 25).

In the 1st stage of the disease, patients often do not seek medical care as they express mild and very few or no signs and symptoms (asymptomatic or pauci-symptomatic patients). Therefore, information and active suspicion of the disease are important to avoid the delay in diagnostic; without active case screening, the diagnosis may be delayed (1, 3, 26).

1.3.2. HAT diagnostics.

Gambiense HAT signs and symptoms are variable, inconsistent, and nonspecific making it confoundable with many other tropical, febrile, and neuropsychiatric diseases. Diseases such as malaria, enteric fever, tuberculous, meningitis, HIV infection can have the same signs or even coexist with gHAT (27). Misdiagnosis with other febrile, neuropsychiatric illnesses and other tropical diseases is frequent (1). Therefore, three step diagnostic approach is used including screening, diagnostic confirmation, and staging (23). Screening tests are based on clinical syndrome and/or serological tests examination. After screening tests, there is a need of performing parasitological confirmation by demonstration of trypanosomes in body fluids because of the lack of specificity of symptoms or serological tests (clinical signs and symptoms are unspecific; serological screening tests detect antibodies only 3–4 weeks after infection and are subject to cross-reactions with other parasitoses). Parasitological confirmation is based on visualisation of trypanosomes at microscope in body fluids sample : lymph node liquid, blood and CSF (3, 23, 27).

Once infection is confirmed parasitologically, lumbar puncture (LP) is required to stage the disease through CSF examination to establish the appropriate treatment. CSF examination is needed too to diagnose relapse after treatment. In case of high clinical or serological suspicion of gHAT, LP and CSF examination could be required as confirmatory diagnostic gHAT test, and in meantime staging the disease in case gHAT is confirmed (3).

i. gHAT screening.

Prior to serological tests development, screening was based on the clinical syndrome and mainly cervical enlargement nodes palpation. Due to the deep weakness in the efficacy and effectiveness of this one, several serological tests were developed and suggested for serological screening: Indirect fluorescent antibody test (IFAT), card agglutination test for trypanosomiasis (CATT), rapid diagnostic tests (RDT), Enzyme-linked immunosorbent assays (ELISA) and Immune trypanolysis tests (3, 28).

a) The Card agglutination test for trypanosomiasis

CATT is a rapid and simple assay for the detection of specific antibodies in patients with gHAT, developed by Magnus; and whose antigen consists of complete bloodstream forms of *T. b. gambiense* variable antigen type LiTat 1.3 (3, 29, 30). The CATT is mainly performed on whole blood (CATT-WB). It can also be performed on plasma (or serum) dilutions (CATT-P) and on blood-impregnated filter paper (micro-CATT) (31-33).

The CATT has some practical inconveniences. The reagents must be kept at 4°C for long-term storage, requiring a cold chain. Vials contain 50 test doses, but, once opened and reconstituted, the reagents can be used only for 1 week when stored between 2°C and 8°C or up to 8h at 37°C. These inconveniences limit the use of the CATT for passive screening in health centres without a cold chain or attended by only a few suspected clinical cases (3, 34).

b) HAT rapid diagnostic tests

HAT RDTs are Rapid individual lateral flow immunochromatographic tests for serological screening of gHAT, available in two formats: a "naked" test strip to dip into a tube containing a mixture of blood, serum or plasma and a buffer or a plastic cassette containing a test strip, with a well to dispense a drop of blood, serum or plasma followed by drops of buffer. If the CATT cannot be performed, it can be replaced by these individual RDTs. Cassette tests can be designed for reading results with portable equipment and wireless data transfer (3).

c) Immunofluorescence assays

Immunofluorescence assays have been used successfully in the control of gHAT in Equatorial Guinea. Serum, plasma, filter paper eluates and CSF can be used. In the test, slides are coated with whole trypanosomes. Immunofluorescence reagents are relatively stable at 4°C, but a fluorescence microscope, large quantities of pure water and electricity are required. Immunofluorescence is therefore suitable mainly for surveillance and reference laboratory diagnosis (3, 27). With the introduction of fluorescence microscopes and portable light-emitting diodes (LEDs), immunofluorescence might be applicable in more remote settings (35-37).

d) Enzyme-linked immunosorbent assays

In Enzyme-linked immunosorbent assays (ELISA) tests the trypanosome antigen is applied to a microplate, blocked with a protein solution (mainly bovine serum albumin or milk powder) before incubation with a diluted sample. As for immunofluorescence, the requirements for sophisticated equipment and large volumes of pure water remain drawbacks for field application of the test. Because of their high sensitivity and specificity, ELISAs, like indirect immunofluorescence, can be used for surveillance and laboratory diagnosis or for surveys to estimate the prevalence of gHAT in certain regions.

e) Immune trypanolysis tests

The immune trypanolysis test for antibody detection involves use of live bloodstream trypanosomes and is therefore restricted to laboratories with the necessary facilities (liquid nitrogen and laboratory animals) to maintain cloned trypanosome populations. The test is based on recognition of the variant (or variable) surface glycoprotein (VSG) epitopes on the surface of live trypanosomes by the corresponding antibodies in the sample, resulting in antibody-mediated complement lysis. Currently, the test is performed with plasma or serum, but a protocol for use with whole blood on filter paper exists. This test is used as a reference test for the presence of *T. b. gambiense*-specific antibodies in quality control of serological testing in the field (3, 38, 39).

In conclusion, gHAT screening in the field is based on CATT and HAT RDT used for mass active screening by mobile or passive screening by health facilities.

ii. <u>Parasite detection.</u>

All clinical and/or serological suspects will undergo parasitological examination as confirmatory diagnostic step. To visualize the parasite in body fluid several diagnostic methods are used : the lymph node aspirates examination (LNA), the thick blood film, the lysed red blood cell (RBC) thick or thin blood smear stained with Giemsa or acridine orange, the micro-haematocrit centrifugation technique (mHCT), also called the capillary tube centrifugation technique (CTC) or Woo test, the quantitative buffy coat test (QBC), the Mini-anion-exchange centrifugation technique (mAECT), and the CSF examination (table 1).

a) The lymph node aspirates examination

The lymph node aspirates examination (LNA) is the most simply and of low cost, and therefore widely used. Its sensitivity is about 59% (43–77%), depending on the focus, the parasite strain, the stage of the disease (sensitivity is higher during the first stage), and the prevalence of other diseases causing lymphadenopathy (3, 27).

b) The thick blood film

The thick blood film is the technique of choice when a centrifuge is not available; other parasites, such as microfilaria and Plasmodium, can be detected. Its preparation needs about 20 µl drop of finger-prick blood. The detection limit in practice is of 5000–10 000 trypanosomes/ml, meaning a sensitivity of 26–35%. It is relatively time-consuming (reading time of 10–15 min per slide) (3, 27).

c) The micro-haematocrit centrifugation technique

The detection limit of the mHCT is of about 500 trypanosomes/ml, meaning a sensitivity of 56% (39–80%). The mHCT is cheap; indeed all the materials necessary to perform mHCT are widely available also, it is moderately time-consuming. Its disadvantages include the requirement for a microhaematocrit centrifuge and some experience in reading the results (3).

d) The quantitative buffy coat test

The QBC combines the concentration of parasites by centrifugation of blood sample in special capillary tube (coated with ethylenediaminetetraacetic acid (EDTA) and acridine orange (AO) and containing a small floating cylinder) with fluorescent staining of Desoxyribonucleic acid (DNA) in the nucleus and the kinetoplast of living trypanosomes by AO. Its detection limit is of < 500 trypanosomes/ml, and the sensitivity of 77% (69–92%). Consumables (capillary tubes) and the cost, sophistication and fragility of material are limiting implementation of this technique, especially in active screening. In addition, dark room and some experiences are needed to read the results.

e) The Mini-anion-exchange centrifugation technique

The large blood volume used in the mAECT allows detection of fewer than 30 trypanosomes/ml when performed on whole blood (mAECT-WB), and fewer than 10 trypanosomes/ml when performed on buffy coat (mAECT-BC), resulting in a high diagnostic sensitivity of 77% (68.8–92.1%) for mAECT-WB and up to 96% for mAECT-BC (3). The mAECT is facing number of limitations: manipulation relatively tedious, time-consuming and require centrifuge, columns. These columns cost a lot of money for large-scale use, laborious production, requiring continuous quality control, no commercial interest as exclusively limited to diagnosis of gHAT. Therefore, mAECT is vulnerable to production discontinuation (3, 27).

f) The Cerebrospinal fluid examination

Regarding the CSF sample, the parasitological test used is the modified single centrifugation (MSC) of CSF is preferred single and double centrifugation of CSF; indeed it's more sensitive and easier to perform (3, 27, 40, 41).

g) Molecular tests

Molecular tests to detect trypanosome DNA or Ribonucleic acid (RNA), include Polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP) and Nucleic acid sequence-based amplification (NASBA) assays. They are restricted to reference centres and research laboratories. They are also used to categorize *Trypanosoma* (sub)species. However, it is not recommended that therapeutic decisions be based solely on their results, as they are indirect and can only identify suspected cases. However, the *trypanozoon*-specific LAMP has been developed into a kit containing all the components to perform the assay out of reference centres and research laboratories, but further evaluations are still needed (3).

Following test on blood are based on concentration techniques: lysis RBC thin and tick blood, QBC, mHCT and mAECT. The most sensitive techniques should be used for parasite detection meaning direct examination of a lymph node aspirate, examination of blood by concentration techniques (preferably the mAECT on buffy coat) and examination of CSF by modified single centrifugation (27). Most techniques, except thick blood films, are based on visualization of trypanosomes by their motility, in fresh sample. Therefore, the time between sampling and examination must be minimal (< 1h) to avoid immobilization and lysis of trypanosomes. In case that the examination must be delayed, keep the sample at 4-8 °C of temperature to keep trypanosomes surviving longer.

h) Diagnostic algorithm

Parasite loads are generally low in gHAT infection and may be below the detection limit of the most sensitive parasitological methods. No single gHAT diagnostic test currently offers satisfactory sensitivity and specificity, meaning that failure to demonstrate parasites does not absolutely exclude infection (27). Diagnostic algorithms combine several tests, including the 3 diagnostic steps of screening, confirmation, and staging. CATT on whole blood or plasma (CATT-WB) or RDT on whole blood performed on clinical suspects (in

health facilities) or performed on people regardless clinical suspicion (during active mass screening) is followed by various parasitological confirmation tests applied either alone or in sequence on neck gland aspirate and/or blood, in view to maximize sensitivity while maintaining acceptable levels of specificity.

Performance, cost and limitations of these different parasitological tests are summarized in table 1.

1.1. Table 1: Performance according to latent class analysis, cost and feasibility of current used gHAT parasitological tests in CATT positive persons (Lutumba *et al* (2006))

Test	**Sensitivity in %(IC95%)**	**Specificity in % (IC95%)**	**Affordability (Cost)**	**Technical difficulty**
LNA	20,8 (13,1-27,0)	99,9 (99,6-100)	Affordable	Simple
the thick blood film	25,9 (18,2-33,7)	100 (99,9-100)	Affordable	Simple but time-consuming
mHCT	57,1 (47,8-66,5)	99,1 (97,1-100)	Relatively less affordable	Sophisticated and difficult to perform on the rural field
mAECT-WB	76,4 (67,6-85,2)	99,0 (96,5-100)	Not affordable for rural population	Difficult to perform by inexperienced persons

iii. Asymptomatic trypanosomes' carriers

Recent evidence is increasingly pointing that asymptomatic carriers harbour trypanosomes in their skin and thus form an anatomical reservoir for *T. b. gambiense*. In fact, xenodiagnotic assays report evidence that those asymptomatic carriers harbouring trypanosomes in their skin have been infective to tsetse flies (42). The diagnosis to date is based on the visualization of the trypanosome under the microscope. Only the examination of a skin biopsy could then provide evidence of a trypanosome infection among those aparasitaemic asymptomatic seropositive individuals. This calls for a review of the definition of gHAT cases to be managed and current policy related to the management of asymptomatic and symptomatic seropositive non confirmed HAT patients (42-46). Indeed, there is a risk that these asymptomatic carriers trigger a resurgence or maintenance of the disease at low level.

iv. Case management

It is recommended that all confirmed gHAT cases must be treated. Treatment aims at reducing the morbidity and the transmission of the parasite to tsetse flies. Until recently, the choice of drug for treatment of gHAT has been guided by the side effects related to the drug toxicity, ease of use, and penetration into the CNS according

to the stage of the disease. Currently, staging of the disease still relies on CSF examination obtained by lumbar puncture (LP) in confirmed cases, an essential step in the process of diagnosis and management of cases (27, 47).

According to WHO recommendations, confirmed gHAT patients with ≤ 5 white blood cell (WBC)/µl and no trypanosomes in the CSF are considered at first or early stage of disease; those with >5 WBC/µl or trypanosomes in the CSF are classified in the second or late stage of disease. The 5-WBC/µl threshold for treatment decision is controversial and some countries used a threshold of 10 WBC/µl (Equatorial Guinea) or even 20 WBC/µl (Angola and Côte d'Ivoire). This initiated to consider 6-20 WBC/µl as an "early second stage" or "intermediate stage", treated with pentamidine to avoid as much as possible the high toxic 2nd stage treatment i.c. melarsoprol, which used to be the first line drug for the 2nd stage patients. Since less toxic drugs have been introduced, the recommended threshold is now 5 WBC/µl (3, 27).

a) Treatment of 1st stage gHAT patients

For gHAT, only one drug is preferable and recommended which is the pentamidine isethionate usually called as pentamidine.

i. Pentamidine

Discovered in 1940, pentamidine, a diamidine, is the drug of choice to treat gHAT patient at early stage of the disease. Because of the risk of hypotension after intravenous (IV) application, the drug is usually administered as deep intramuscular injection, at the primary health-care (PHC) level; 4 mg / Kg, once-daily for 7 days. Despite some side effects such as site pain and transient swelling, abdominal pain, diarrhoea, nausea, vomiting, hypoglycaemia (5 – 40%) … reversible for the most of them, this drug is generally well tolerated by patients. Also hyperglycaemia was reported (5%) with very rarely persistent manifestation of diabetes (48).

Treatment failures to pentamidine are rarely reported, however they increased when pentamidine was administrated to "intermediate or early 2nd stage" patients, which indicates that failures are likely related to misdiagnosed stage 2 disease rather than drug resistance. Indeed, pentamidine induces low levels of drug detected in the CSF to be used for the 2nd stage of the disease (48-50). Balasegaram *et al* (2006) reported a treatment failure of 4% among HAT patients with ≤ 5 WBC / µl (and no trypanosomes) in the CSF vs 12% among the ones with 6 – 10 WBC / µl (and no trypanosomes) in the CSF (50)

b) Treatment of 2nd stage gHAT patients

All drugs belonging to second stage are also effective to the first stage according to the availability of less toxic drugs.

i. The Melarsoprol (known as Arsobal)

Introduced in 1949, melarsoprol is an arsenic derivative used to treat gHAT patients at 2nd stage of disease.

The administration is by intravenous route, 2.2 mg/kg once a day, for 10 days.

It has many adverse effects, the most serious of which is reactive encephalopathy (encephalopathic syndrome). It's probably of immune origin, occurring variably in an average of 4.7% (2% - 10%) of *T. b. gambiense* patients, with a case fatality rate of approximately 50%. Melarsoprol-associated encephalopathic syndrome (ES) is mostly described as unpredictable and abrupt development of convulsions and intense agitation, followed by coma. These two signs could occur separately, and mental (psychotic) changes could be observed too. ES may occur at any time-point after the first melarsoprol administration and up to 30 days after the last one, with a median and peak occurrence around day nine of treatment.

Due to high rate of severe side effects, some of which life-threatening, hospitalisation is mandatory during treatment with melarsoprol (47, 48, 51, 52).

Melarsoprol treatment failures began to appear in the 1990s and rapidly spread in various foci (49). Robays *et al* (2008) reported a failure rate to melarsoprol of 19.5% (53). Pentamidine and melarsoprol cross-resistance has been reported (54). (55).

ii. The Difluoromethylornithine known as eflornithine or DFMO

Approved in 1990, the Difluoromethylornithine (DFMO) (Eflornithine ®) (56, 57), a trypanostatic that inhibits ornithine decarboxylase, an enzyme essential for cell multiplication and differentiation (58) showed a clearly reduced mortality and cumulative incidence of relapses, while being as effective as melarsoprol with a superior safety profile. It was therefore recommended as first line treatment versus melarsoprol for second stage gHAT (48, 59).

It is administrated intravenously, 100 mg/kg body weight, 4 times a day (at intervals of 6 h) for 14 days as short infusions.

Drug side effects are frequent, like other cytotoxic drugs for the treatment of cancer (including convulsions, gastrointestinal symptoms like nausea, vomiting and diarrhoea, bone marrow toxicity leading to anaemia, leukopenia, and thrombocytopenia), with occurrence and intensity that increase with the duration of treatment and the severity of the general condition of the patient. Generally, eflornithine side effects, when administrate to treat gHAT, are reversible after the end of treatment.

Administration of DFMO in monotherapy was very expensive, complex to be administrated (requiring 56 short infusions over two weeks, sufficient and trained human resources to maintain adequate round-the-clock nursing care), asking for a so constraint logistic (kit of 1 m^3 of volume including all necessary ancillary materials for just 2 patients, provided by WHO). Although Eflornithine treatment cost higher (997.14 USD) than melarsoprol (708.03 USD) to treat 2nd stage patient, the fact that it saves more lives make it more cost-

effective (60). Unfortunately, considering those difficulties to administer eflornithine in resource-poor settings, melarsoprol continued to be the first-line treatment in some areas.

This latter and the fact that eflornithine monotherapy was facing a high risk to develop drug resistance, made it crucial to identify drug combination regimens as soon as possible to protect it, especially as the number of compounds active against *T. brucei* spp. with acceptable toxicity were limited (48).

iii. The Nifurtimox

Nifurtimox, that was introduced for treatment of Chagas disease (*Trypanosoma cruzi*) in the late 1960s, has been successfully used for the treatment of melarsoprol-refractory gHAT infection. It has been used for compassionate treatment, 5 mg/kg and 7 mg/kg 3 times a day (every 8 h) for adults and children, respectively, for 14–21 days. Bisser *et al* reported an efficacy of 65.2% (61).

Nifurtimox is generally not well tolerated, and only about one-third of the patients remain free from adverse drug reactions. Nifurtimox adverse reactions are generally not severe, very rarely fatal, dose and therapy duration related and rapidly reversible after discontinuation of the drug. They include gastrointestinal disturbances with nausea, abdominal pains and vomiting (very frequent), and neurological adverse reactions with general convulsions, tremor or agitation, and occasionally the development of peripheral polyneuropathy and generalized skin reactions events (48).

iv. The Nifurtimox-Eflornithine Combination Treatment

Nifurtimox was selected to be used (15 mg/kg per day for 10 days administrated by oral route) in association with Eflornithine, administrated intravenously (Nifurtimox – Eflornithine Combination Treatment, NECT in acronym), or in association with melarsoprol. Priotto *et al* (2006) reported cure rate of 44.4%, 78.9% and 94.1% treating 2nd stage gHAT patients with Melarsoprol – Nifurtimox combination, Melarsoprol – Eflornithine combination and Nifurtimox – Eflornithine combination treatments respectively. The cure rates were significantly higher with Nifurtimox – Eflornithine combination treatment ($p = 0.003$) and Melarsoprol – Eflornithine combination ($p = 0.045$) than with Melarsoprol – Nifurtimox combination. Adverse events were less frequent and less severe with Nifurtimox – Eflornithine combination treatment, resulting in fewer treatment interruptions and no fatalities compared to melarsoprol-nifurtimox and melarsoprol-eflornithine combination treatments (58, 62). The efficacy was better in the combination arms compared to the monotherapies. However, combinations containing melarsoprol resulted in very high frequencies of severe adverse drug reactions and enrollment was terminated early because of excess toxicity in both melarsoprol-containing groups (61-63).

The nifurtimox - eflornithine combination therapy (NECT) was introduced in 2009 (64). It considerably simplifies the administration of eflornithine monotherapy by reducing the duration of treatment and the number

of intravenous infusions: 200 mg/kg i.v. short infusion every 12 h for 7 days for eflornithine associated to nifurtimox 15 mg/kg orally per day (5 mg/kg every 8 h) for 10 days. Therefore, the number of eflornithine infusions was reduced from 56 to 14, the hospitalization time shortened by one third (from 14 to 10), the staff and logistic resources required was reduced and a positive effect against development of drug resistance could be predicted (58).

Priotto *et al* (2009) reported that, among patients aged of 15 years and older, NECT was non-inferior to eflornithine monotherapy (91.6% and 96.5% cure rates at 18 months follow up for Eflornithine and NECT respectively, with a difference of – 4.9%, one-sided 95% CI – 0.3; p<0·0001; with very similar results showed by per-protocol analysis) and presents safety advantages (even if drug related adverse events were frequent in both groups, 28.7% of patients in the eflornithine group versus 14.0% in the NECT group experienced major (grade 3 or 4) reactions) (63). Other studies under field condition, reported similar NECT cure rates in pregnant women, breast-feeding women and children. Regarding safety, a better tolerance to NECT was observed in children who, on average, experienced fewer adverse events than adults (65-67). The adherence to treatment varied slightly amongst the patient groups, with the lowest adherence in pregnant women group (nifurtimox 79% & eflornithine 93%) and in small children below 5 years of age (nifurtimox 89% & eflornithine 86%) (66). NECT cost (387.68 USD) less than Eflornithine administrated itself (60). Considering all these advantages, tolerance to the various drugs, and the potential protective effect against the emergence of resistant parasites, NECT was suitable for first-line use to treat second stage patients. Furthermore, due to the advent of less toxic drugs to treat second stage cases, such as Eflornithine or NECT, melarsoprol is no longer used to treat gHAT cases, unless it is the last choice, as the last treatment option for relapsed cases.

c) Treatment option for both 1st and 2nd stages of gHAT

i. Fexinidazole

Fexinidazole had been in preclinical development in the 1970s as an antimicrobial agent, but its development was abandoned. It was rediscovered and revived more than 20 years later by DNDi through a systematic review and profiling of different nitro-heterocyclic compounds (68). Kande *et al* (2018, 2021) assessed the efficacy and safety of fexinidazole (a 2-substituted 5-nitroimidazole with proven trypanocidal activity) versus NECT in patients older than 15 years, able to ingest at least one complete meal per day (as fexinidazole required ingestion of meal to be effective), diagnosed with late-stage gHAT (defined as patients with trypanosomes found in blood or lymph node fluid and ≥ 20 WBC / µl or trypanosomes in the CSF) (69) and with stage 1 or early stage 2 gHAT (stage 1 defined as patients with trypanosomes in blood or lymph, no trypanosomes and ≤ 5 WBCs / µl in the CSF and early-stage 2 defined as patients with trypanosomes objectified in blood or lymph, no trypanosomes and 6 – 20 WBCs / µl in the CSF) (70). Oral fexinidazole was given once a day (1800 mg

daily: days 1–4, and 1200 mg daily: days 5–10). Oral nifurtimox was given three times a day (15 mg/kg per day: days 1–10) with eflornithine twice a day as 2 h infusions (400 mg/kg per day: days 1–7).

Success rates at 18 months (patients being alive, having no evidence of trypanosomes in any body fluid, not requiring rescue medication, and having ≤ 20 WBC / μl in the CSF) treating gHAT late stage were higher than expected in both treatment groups; 91.2% in the fexinidazole group (89% expected) and 97.6% in the NECT group (94% expected) with a difference between groups (–6.4%, 97.06% CI –11·2 to –1·6; p=0·0029) within the predetermined 13% margin of acceptable difference (p=0.0294). There was no difference in the proportion of patients who experienced treatment-related adverse events between the two groups (81% in the fexinidazole group vs 79% in the NECT group), no treatment discontinuations related to treatment (1% in the fexinidazole group, and 2% of patients were subject of temporary interruption in NECT group), and no difference in death proportion rates between the two groups (3% of patients died during the study in the fexinidazole group vs 2% in the NECT group). The following adverse events were the most frequently reported: headache, vomiting, nausea, and insomnia (that represent the largest difference between groups; 28% vs. 12%) (69, 71).

In patients with stage 1 gHAT, at 18 months, Fexinidazole treatment was effective in 98% (95% CI: 94.7 – 99.4) of patients (70), results non-inferior to the historical results of pentamidine among patients with stage 1 gHAT estimated at 96.0% (94.0–97.4%) (50) and exceeding expectations (80%) (70). The Fexinidazole was effective in 98% (87.1 – 99.9) "early-stage 2" patients; the comparison to historical results regarding NECT was not possible as the populations treated with NECT were different, but the results were exceeding expectations (80%) (70). In total, 93% of all patients present treatment emergent adverse events during the study; equally in stage 1 and "early-stage 2" gHAT patients (93%), and most of them were mild or moderate, including headache and vomiting (70, 71).

Additional studies in children aged ≥ 6 years and ≥ 20 Kgs showed that Fexinidazole was as effective and safe than in adults (72, 73), and therefore we can assume that fexinidazole is effective and safe for the treatment of *T. b. gambiense* infection compared with pentamidine for early-stage patients and to NECT for late-stage gHAT patients in adults and in children ≥ 6 years and ≥ 20 Kgs. In addition to this, Fexinidazole has number of advantages, which positively counterbalances some loss of efficacy, compared to NECT, which moreover is within the acceptable fixed margin. Fexinidazole is effective in both early and late stages gHAT, therefore lumbar puncture can be avoided. It involves a simplified short-course oral regimen, given with a simple, locally adapted meal, it could be administrated at a PHC facility as opposed to NECT that needs to be administered in a hospital setting by trained personnel, which is not optimal, given that patients often live in remote areas with few health resources. Fexinidazole is currently the only one available oral monotherapy developed and tested to treat gHAT patients. As such, it circumvents all potential complications associated with intravenous catheter use, with positive financial effects at both patient level (no mandatory hospitalization, no need to travel to

specialized healthcare centre; reduced interruption of employment …), and healthcare level (fewer medical resources required, simplified logistic …). Oral treatment could benefit patients who are unwilling to be treated in hospital, who could receive homebased treatment. Such approach could potentially increase accessibility to treatment, reaching more people in need. However potential concerns with respect to adherence are present, given that (a) fexinidazole must be administered during or after a main meal to achieve effective concentrations, as bioavailability is substantially compromised in the unfed state (74), (b) the dosing schedule of 10 days is relatively long for an oral treatment, (c) the number of tablets changes midway through treatment, and (d) nausea and vomiting are frequent side-effects. A study including a sub-cohort on homebased treatment is ongoing to help assess these difficulties (69). Fexinidazole cost less than NECT (< 50 USD) (60).

Based on all scientific evidence, the recommendations from the guidelines development group that included individuals with recognised expertise in the field of treatment of gHAT, public health, and national control programmes, WHO elaborated "interim guidelines for the treatment of *gambiense* human African trypanosomiasis" to be observed for gHAT case management (55, 73). Those guidelines took in account the following basis:

- The safety and efficacy of fexinidazole in the < 6 years or < 20 Kgs group has not been established in clinical trials;
- The guideline development group suggests using fexinidazole in favour of pentamidine in patients with first stage gHAT: Fexinidazole was as effective as pentamidine, and the balance of desirable and undesirable effects appears to favour fexinidazole;
- Although Fexinidazole cure rate was found to be in acceptable margin of difference with NECT, with number of advantages on Fexinidazole side compared to NECT, deep analyses revealed that the treatment failure rate at 18 months in patients with 2nd stage gHAT and with ≥ 100 CSF WBC / µl was significantly higher for fexinidazole, 13.1% (95% CI: 8.3 – 19.4), than for NECT, 1.3% (95% CI: 0.0 – 6.9) and by contrast to other groups: 2.0% (95% CI: 0.2 – 6.9), in group with < 100 CSF WBC / µl, where treatment failure to NECT was of 4.1% (95% CI: 0.5 – 14.0), 2.4% (95% CI: 0.1 – 12.9), in group ≤ 15 years of age group at early-stage 2 gHAT, and 1.8% (95% CI: 0.1 – 9.6), in group of children of 6-15 years at 2nd stage;
- Fexinidazole is given orally for 10 days and fexinidazole tablets should be taken with a meal.
- The following recommendations have been formulated regarding treatment with Fexinidazole:
- To prescribe fexinidazole, the prescriber must have confidence that food is available to the patient and will be eaten directly before the daily drug administration.
- Directly observed treatment (DOT): each intake of fexinidazole must be supervised by a trained health staff who must ensure that the patient is in a fed condition.

- Outpatient administration (under daily supervision) can be decided in consultation with the patient, his/her family and clinicians, taking into account the following factors:
  a) convenience to the patient and the family (e.g. distance and costs);
  b) development of side-effects interfering with treatment compliance;
  c) existing comorbidities; and
  d) capacity of the healthcare system for supervised administration as an outpatient.
  e) Hospitalization should be mandatory in the following cases:
  f) patients with psychiatric disorders;
  g) children with body weight < 35 kg;
  h) patients with ≥ 100 WBC treated (exceptionally) with fexinidazole; and
  i) risk of poor compliance with treatment.

ii. Pafuramidine (DB289) and Acoziborole

Since there were only few therapeutic options available to treat gHAT, to enlarge the number of drugs active against gHAT, number of new drug targets were identified and numerous compounds with promising activity in preliminary tests identified including pafuramidine (DB289) and SCYX-7158 (acoziborole) (48, 57, 68, 75, 76). Regarding DB289, despite promising initial results, the identification of acute renal toxicity led to the clinical development suspension (57), while researches on acoziborole (SCYX-7158), a promised single dose oral cure drug for stage 2 gHAT, are still ongoing (77, 78).

d) Strengthens and limitations of different gHAT drugs

Strengthens and limitations of drugs used to treat gHAT cases are summarized in table 2.

1.2. Table 2: gHAT drugs in current use

Drugs	indication and administration	limitations
Pentamidine	The drug is administrated at the primary health-care (PHC) level. The drug is generally well tolerated. Treatment failures are rarely reported. Indicate for the 1st stage gHAT patients.	Risk of hypotension after intravenous (IV) application. Risk of hyperglycaemia

Drugs	indication and administration	limitations
Melarsoprol	Treatment of 2nd stage gHAT patients.	Serious adverse effects. Fatality rate of 50% approximately in case of encephalopathy. High failure rate (19.5%). Currently no longer used to treat gHAT cases, unless if it is the case of relapse from other drugs.
DFMO	1st line treatment for 2nd stage gHAT.	Frequent side effects reversible after the end of treatment. Very expensive cost. Complex to be administrated, requiring 56 short infusions over two weeks. High risk to develop resistance.
Nifurtimox	Second stage of the disease.	Compassionate used in absence of any other drug
Nifurtimox-Eflornithine combination therapy (NECT)	1st-line use to treat 2nd stage patients vs DFMO.	Better tolerance and posology
Fexinidazole	Effective in both stages gHAT.	Oral treatment

e) Treatment WHO guidelines for gHAT

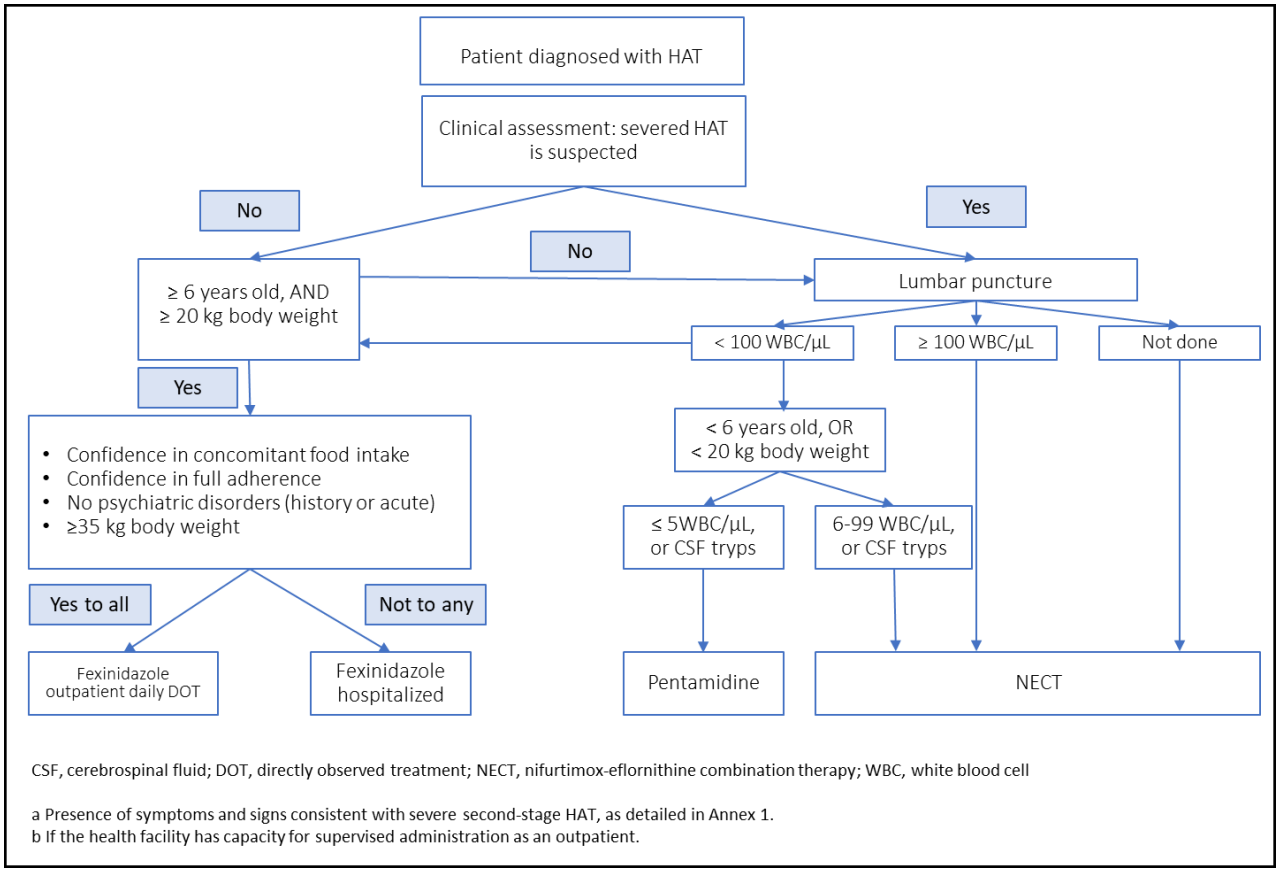
For the treatment, WHO interim guidelines formulate recommendations (55, 73) that could be summarized as follows (Fig 2):

- For children aged < 6 years or body weight < 20 kg, LP is required and pentamidine will be used in first stage and NECT in second stage according to LP examination results.
- In case of absence of clinical suspicion of severe 2nd stage gHAT, among patients aged ≥ 6 years and weighing ≥ 20 kg, a LP can be avoided and fexinidazole preferentially given on condition of having high confidence in appropriate follow-up to detect relapses early. The following symptoms and signs, correlating with severe meningoencephalitic gHAT and assessable in peripheral health facilities, could be

used for selection of such patients: mental confusion, abnormal behaviour, logorrhoea, speech impairment, anxiety, tremor, motor weakness, ataxia, abnormal gait, abnormal movements, and seizures. Sleep disorder alone can't determine the need for a LP as even if very common in severe gHAT, it's frequent too in non-severe gHAT.

- Patients aged ≥ 6 years and weighing ≥ 20 kg clinically suffering from severe gHAT will be subjected to LP in order to decide between Fexinidazole (≤100 CSF WBC / µl) or NECT (≥ 100 CSF WBC / µl)
- Patients aged ≥ 6 years and weighing ≥ 20 kg who reject or do not tolerate fexinidazole may need a LP to decide between pentamidine and NECT.
- Patients who require LP stratification but do not receive LP, and those whose LP results are unreliable, will be treated preferentially with NECT.
- Pregnant women: former WHO recommendations apply and Fexinidazole can be given after the 1st trimester.

Recommendations for anti-trypanosomal treatment during pregnancy and lactation are based on clinical practice rather than on solid evidence. Pentamidine and fexinidazole can be given after the first trimester. Melarsoprol, eflornithine and nifurtimox are all theoretically contraindicated during pregnancy; their use and treatment timing (stage of pregnancy) depend on the general condition of the mother. If the general condition of the pregnant woman is moderately or severely altered, treatment with fexinidazole, eflornithine alone or NECT must be administered with the main objective of saving her life. After delivery, the newborn should be examined clinically and checked for the presence of circulating trypanosomes in the blood. Breastfeeding should continue during gHAT treatment (55).



1.2 Figure 2. Algorithm of WHO recommendations for the management of gHAT (55)

Treatment outcome assessment

The meeting of the WHO expert committee on the control and surveillance of African Trypanosomiasis held in Geneva, 21-27 November 1995, recommended patients' post-treatment follow-up at 3 and 6 months after the completion of treatment and thereafter every 6 months, up to 2 years to assess cure or treatment relapses or failure. At each of these appointments, the treated patients are examined clinically; and their blood sample and CSF examined for trypanosomes and CSF WBC count (2).

Considering the low failure rate to pentamidine and NECT used as 1st line treatment, and considering the low compliance with follow-up in practice (75), systematic follow-up after treatment is no longer recommended in routine; patients are encouraged to present themselves when clinical symptoms of gHAT do appear. At the controversy, for clinical trials of new drugs or new treatment regimens, treatment outcome assessment is required through an 18-month follow-up instead of 24. In fact, the proportion of patients with post-treatment follow-up data available is much higher at 18 months than at 24 months, and this even in studies with active follow-up. Also, most of patients who relapse within 24 months of treatment have already relapsed within 18 months. Therefore, efficacy assessment based on 18 months post-treatment follow-up data would provide a good estimate of the efficacy of the drug under investigation, particularly in comparative studies.

Consequently, 18 months has been recommended as the time for the final efficacy assessment (test-of-cure visit) in clinical studies (76).

Since fexinidazole is a new drug and given the risk of poor adherence to treatment and/or the required concomitant intake of food essential for absorption of the drug, the interest of systematic follow-up is high. In patients treated with fexinidazole, relapses may occur late, even 12 to 24 months after treatment. It is therefore recommended that these patients be asked to present for a general examination at 6, 12, 18, and 24 months, or at any time that symptoms may recur. If signs or symptoms indicate a possibility of relapses, laboratory tests of body fluids, including CSF, should be performed to detect trypanosomes and/or count CSF WBC (55).

Relapses is defined by (55)

- the presence of trypanosomes in any body fluid or tissue at any period of follow up (0-4 months, 6, 12, 18 or 24 months
- 6 months post-treatment (5–9-month window):
    - 6–49 CSF WBC/µl means an uncertain evolution that call for a new follow-up at 12 months or a rescue treatment decided by the clinician in presence of clinical features suggesting relapses.
    - ≥ 50 CSF WBC/µl of CSF is considered as a relapse inducing a rescue treatment.
- 12 months post-treatment or later (10–24-month window):
    - ≥ 20 CSF WBC/µl means relapses and induce a rescue treatment.

In case of relapses to

- Fexinidazole, NECT should be given,
- Pentamidine, fexinidazole or NECT should be administered depending on the patient's age/weight and the CSF WBC count,
- NECT, the 1st rescue treatment should be NECT-long (400 mg/kg per day of eflornithine in two infusions for 14 days, and nifurtimox given for 10 days, exactly as in the NECT schedule), and alternatively eflornithine monotherapy (100 mg/kg every 6 h for 14 days), or fexinidazole if age/weight appropriate and WBC in CSF < 100 cells,
- To all above rescue treatments, then melarsoprol should be considered as a last treatment option due to its toxicity.

2. gHAT control and elimination

2.1. Concepts definition

Disease control means reduction of disease incidence, prevalence, morbidity and/or mortality to a locally acceptable level as a result of deliberate efforts while continued interventions are required to maintain the

reduction. The "elimination as a public health problem (PHP)" is defined by achievement of measurable targets set by WHO in relation to a specific disease. When these are reached, continued action is required to maintain the targets and/or to move toward interruption of transmission. Documentation of elimination as a PHP is called validation. Elimination (interruption of transmission) is defined by reduction to zero of the incidence of infection caused by a specific pathogen in a defined geographical area, with minimal risk of reintroduction, as a result of deliberate efforts; continued action to prevent re-establishment of transmission may be required. Documentation of elimination of transmission is called verification. Eradication is permanent reduction to zero case of the worldwide incidence of infection caused by a specific pathogen, as a result of deliberate efforts, with no risk of reintroduction. Documentation of eradication is termed certification by WHO (77). Member States have to submit a dossier to claim the certification of elimination by WHO.

The gHAT was targeted for elimination as PHP by 2020, and for interruption of transmission by 2030 (3, 78-80). At the global level (meaning continental level), two primary indicators were used to monitor the progress towards the 2020 goal of gHAT elimination as a PHP: (A) the number of cases reported annually, the target being < 2,000; and (B) the area at risk reporting ≥ 1 case/10,000 people/year, calculated over 5-year periods, the target being a reduction of 90% (i.e. 638,000 km2) by 2016–2020 compared to the 2000–2004 baseline (i.e. 709,000 km2). Three secondary indicators, not linked to specific targets, were also monitored: (a) the disease geographic distribution, (b) the at-risk population, and (c) the coverage of the at-risk population by control and surveillance activities. At country level, following indicators were considered: < 1 case/10,000 inhabitants/year (averaged over a 5-year period) reported in each health district of the country, in conjunction with adequate, functional control and surveillance (meaning intensity and effectiveness of gHAT control and surveillance activities, including active and passive case finding, vector control and African animal trypanosomiasis (AAT) control, assessed as adequate, insufficient, or absent). The choice of the health district as the basic geographical unit of analysis and reporting is in line with many other NTDs, including visceral leishmaniasis, trachoma and lymphatic filariasis (4).

2.2. gHAT disease control

The gHAT is considered as an anthroponosis, where animal would play a minor role. Therefore, gHAT control has relied heavily on active and/or passive case detection and treatment, complemented by vector control (3, 23, 81). Clinical signs and symptoms related to gHAT are relatively nonspecific and can mimic other tropical diseases like malaria, enteric fever, tuberculous meningitis, or HIV infection which could even coexist with gHAT (27). Patients with gHAT may remain asymptomatic or have only mild symptoms for many months before seeking care, and once care is sought, the low index of suspicion by primary health care workers and difficulties in observing the parasite frequently result in diagnostic delay (3). In the meantime, they progress to the advanced stage of the disease and spread it among their relatives and community members, having been

infectious to tsetse flies for a prolonged period. Therefore, the case detection approach is most effective when cases are identified early. For these reasons, active case detection by mobile teams, screening the population at risk at regular intervals to find cases as earlier as possible, has been for long time the main gHAT control strategy (3, 26, 27).

Active case detection of gHAT is carried out by specialized mobile teams of 7 to 9 members going from village to village and screening the entire villages or urban neighbourhoods' populations at risk. The screening was, in earlier times, based on clinical suspicion (palpation of the neck glands). Now it is based on CATT as serological screening test prior to confirmatory parasitological examination (82). This systematic case detection and treatment suggested by the French military surgeon Eugène Jamot (1879–1937) was applied with success during colonial times resulting in the fact that the disease was under control, almost eliminated at the dawn of 1960s corresponding with the independence period of the most of affected countries (83). Active case detection was successful in several countries to control the last outbreak in the 1990s (84), as the main strategy (85, 86) or associated with vector control activities (87). However, the success of the active case detection, even maintained over many years, can be torpedoed by some factors such as the limited sensitivity of the diagnostic methods and low attendance rate of population (1, 82). The mandatory nature of the gHAT screening of colonial times that allow screening almost all people in the targeted regions was abolished after independence. High toxic drugs, lack of confidentiality, lumbar puncture and financial barriers have been among factors that undermined population attendance to active screening and removing them could improve the effectiveness of the active case detection. Also when the prevalence of gHAT decreases the attention in the population drops and with it the decrease in attendance to screening activities (26, 84).

The maintenance of some foci despite the effective active screening suggests existence of animal or asymptomatic reservoirs (42, 88, 89).

With the decreasing of gHAT prevalence, the active case finding through mass campaigns by large mobile team composed with 7 to 9 persons called as the truck based mobile teams become less cost-effective (26). Therefore, targeted door-to-door surveys may become an attractive alternative to mass screening (90-92); and integration of gHAT control and surveillance activities through passive screening in the PHC system will also become crucial (1, 26).

Passive case detection is complementary to active case detection. Patients presenting themselves to PHC facilities with signs or symptoms suggestive of gHAT are screened and tested for the latter. The passive case detection allows to find at least half of all detected cases (82). Unfortunately most of the patients detected during passive screening are at advanced stage of the disease, due to the significant delay from patients and health system (26). Therefore, passive screening is less contributing to reduce the human reservoir which is mainly based on early detection (3, 85). The gHAT passive screening is facing several limitations. Indeed,

only a few facilities can be equipped with the CATT because of the lack of cold chain non-existing in remote areas where gHAT is most prevalent. Low attendance rates at health centres (93) may limit the use of the 50 tests CATT supplied vials because once reconstituted the CATT test must be stored for less than a 1 week at 4–8 °C (94). The lack of electricity cannot allow to perform mHCT, mAECT, QBC, MSC of CSF. These tests also require equipment and trained persons. In fact, health centers in remote areas and isolated areas where gHAT typically occurs are equally fragile, understaffed, and less equipped (94, 95). Also 2nd gHAT cases required hospitalisation setting and trained personnel to be treated.

To overcome these limitations, tools and approaches have been suggested, such as the thermostable format of CATT test (provided in 10 tests vials), the individual rapid serodiagnostic tests (RDTs), the micro-CATT, where dried blood sample on filter paper could be sent to a health facility performing CATT, the referral of suspects or unconfirmed cases of gHAT to well-equipped facilities to be subjected to the most sensitive diagnostic techniques (3, 95, 96). Out these technical issues, integrating gHAT control and surveillance into PHC system call to address some additional issues like ensuring availability of trained, competent and motivated personnel, removing or reducing financial barriers, providing friendly-use and integrated screening / diagnostic procedures and tools (97). Integrating gHAT screening activities into first-line health facilities could improve, aside active screening by mobile teams, the coverage of the population at risk. Especially since PHC facilities are always available and close to the community (98). In addition, fexinidazole as an oral administration drug will facilitate gHAT integration in PHC services.

Vector control activities has not played a major role in gHAT, as it was considered too expensive and difficult to deploy in the resource-poor settings of gHAT foci (96) and its added value to gHAT active screening was discussed for a long time. However, evidence from modelling, historical investigations, and practical interventions has demonstrated that vector control can play a significant role in the control of gHAT (96, 99, 100). In absence of vaccine or chemoprophylaxis to prevent gHAT, vector control seems to be the only prophylactic measure to protect people against the infectious bite of the tsetse flies (81). It is perceived that it can be particularly effective in times of low endemicity where it may be more cost-effective; and at this time xeno-monitoring using LAMP kits could help to identify potential sleeping sickness transmission sites (96, 101).

The choice of vector control method depends on the local environment and epidemiology, the human and financial resources available and the potential ecological impact. These could include clearing of vegetation, but in gHAT foci, they are mainly based on traps or insecticide-impregnated screens made of blue and black fabrics. In fact, tsetse flies are visually attracted by the royal phthalogen blue. The fabrics used should be chosen according to the spectrum of reflectance also (3). In view to reduce the cost towards simpler and cheaper alternative for vector control, suitable for gHAT foci, insecticide-treated large targets were modified in tiny

targets. It was found that the small square of blue cloth flanked by a similar sized piece of black netting (tiny target) was more cost-effective than traps or large targets (81). Other vector control methods include "sterile insect technique" which appears unfeasible continent-widely (96). Serological test based on tsetse-saliva biomarkers is suggested to monitor the effectiveness and sustainable impact of tsetse control interventions (96, 102).

The persistence of some gHAT foci despite case detection activities including active screening and even vector control well conducted raise the unaddressed question of animal and asymptomatic reservoirs. If the role of animals as reservoir is considered as limited and need more evidence, the role of asymptomatic as reservoir that could play important role in maintenance or resurgence is now more documented and policies in regard would need to be updated.

Considering all that above, the disease control programme with mobile teams, specialized health facilities, and the integration of gHAT control and surveillance in PHC system have to coexist in adapted manner taking in consideration the epidemiological situation and capacities (98, 103).

2.3. gHAT elimination and eradication

The gHAT is one of NTDs targeted for elimination by 2030 while it was targeted for elimination as PHP in 2020. It requires continuous action to maintain achievements until elimination (end of transmission) and from there until eradication of the disease. The gHAT is a localized, focal disease, a focus being defined as "a area of transmission to which a geographical name is given (locality, region, or river)". Most of foci are subject to migration of humans and/or flies from neighbouring foci (open foci). Therefore, surveillance post-elimination interventions is required especially when there is still a risk of resurgence and/or reintroduction of infection from outside the focus, which indeed is still an issue even if the disease is drop down from these nearby foci (3).

Whether elimination as PHP or interruption of transmission, continued action is required to maintain targets, make progress forwards or prevent re-establishment of transmission. Continued action corresponds to classical control methods that include active case detection, passive case detection and targeted vector control implemented in selected foci. Passive screening will be expanded to selected sentinel sites. All these methods will be combined in a manner determined by the intensity of disease transmission in the focus (Figs 3 & 4) (78). When gHAT is considered to be eliminated in a focus, disease surveillance ("post-elimination surveillance) will be put in place to detect any disease re-emergence. Apart the surveillance in sentinel sites, different tools and strategies such as micro-CATT and enzyme linked immunosorbent assay (ELISA)/ *T. b. gambiense* on filter paper (33), immune trypanolysis test (TL) on frozen plasma sample (38, 39), the *T. b. gambiense* inhibition ELISA (g-iELISA) on fresh or dried plasma sample (104) have been assessed and suggested for this surveillance. Due to TL's biosafety issues and technological requirements, g-iELISA could

be used (39). Apart from the need for tools and strategies to identify timely gHAT re-emergence, elimination sustainability is facing number of challenges such as drug resistance and limited accessibility to the gHAT at-risk population (105)

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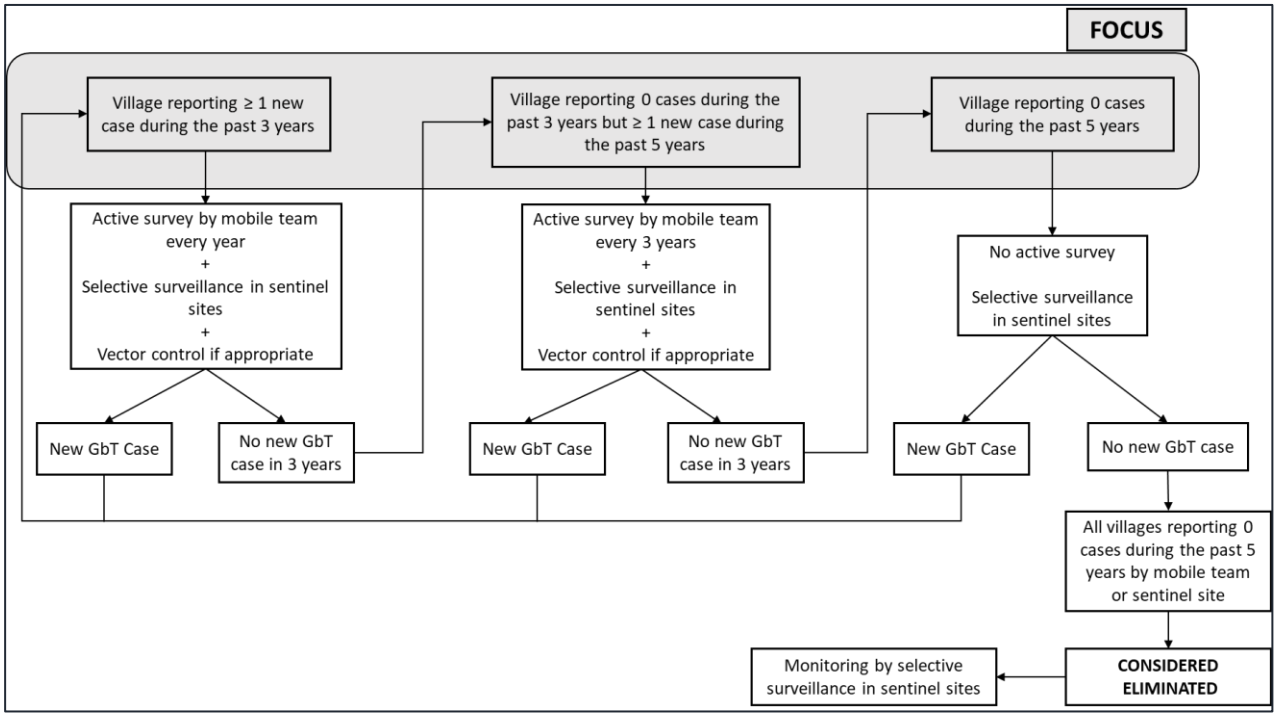

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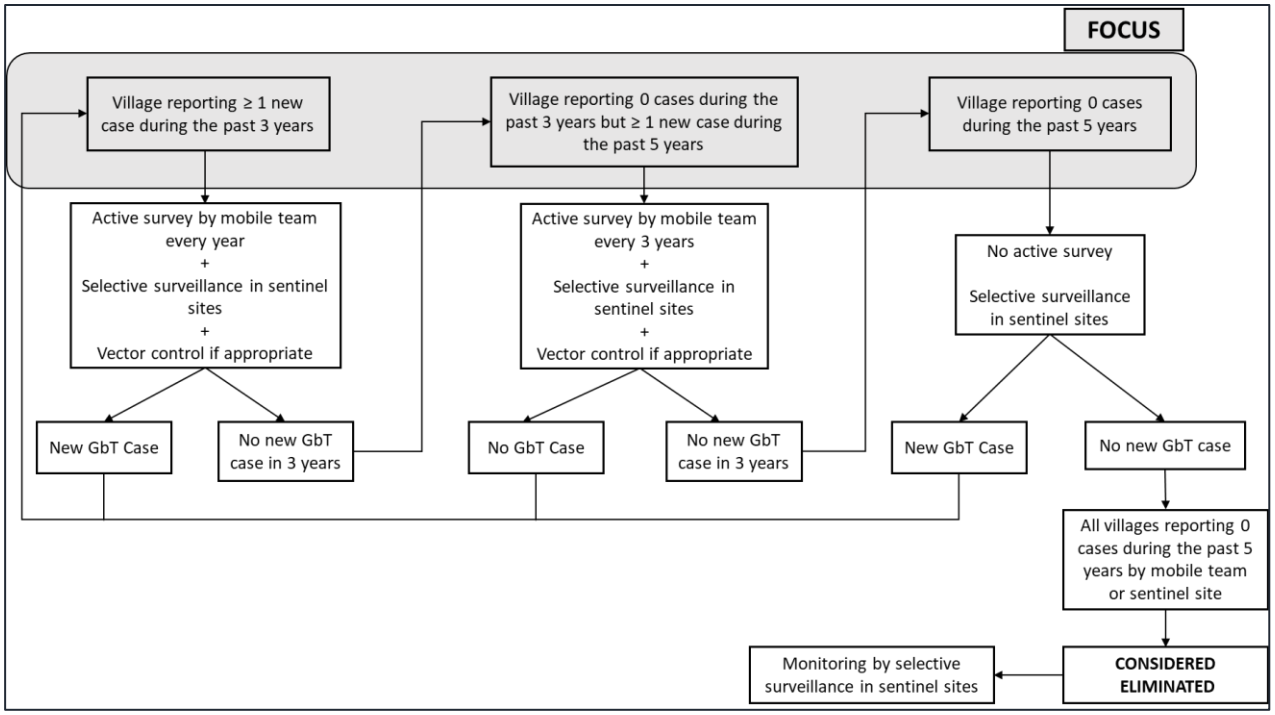

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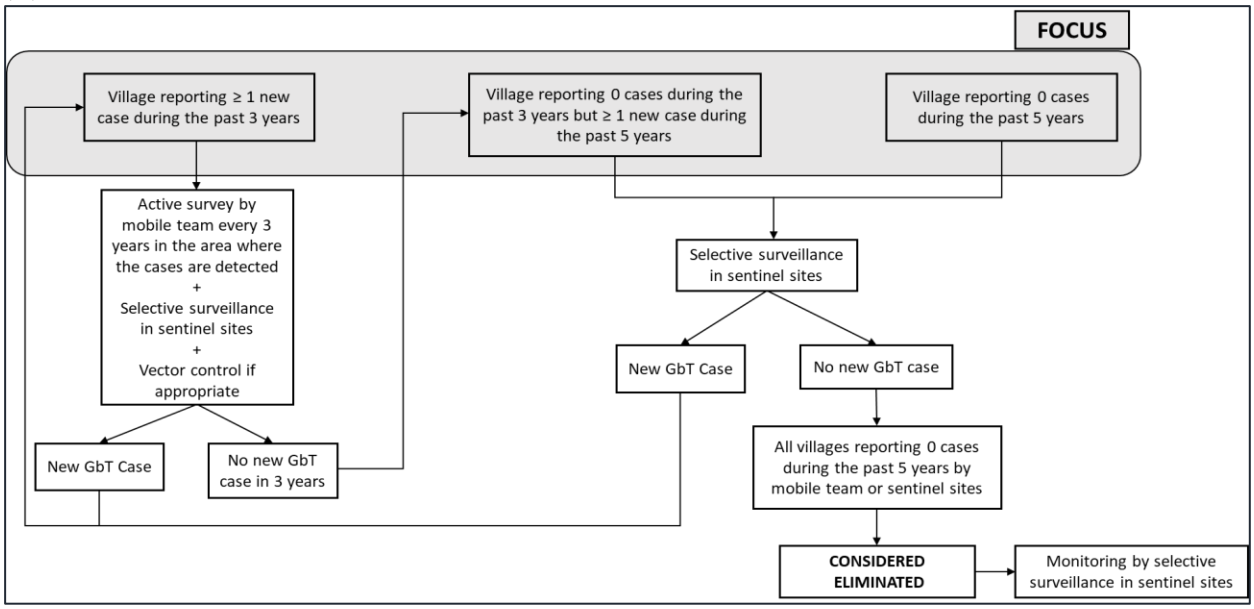

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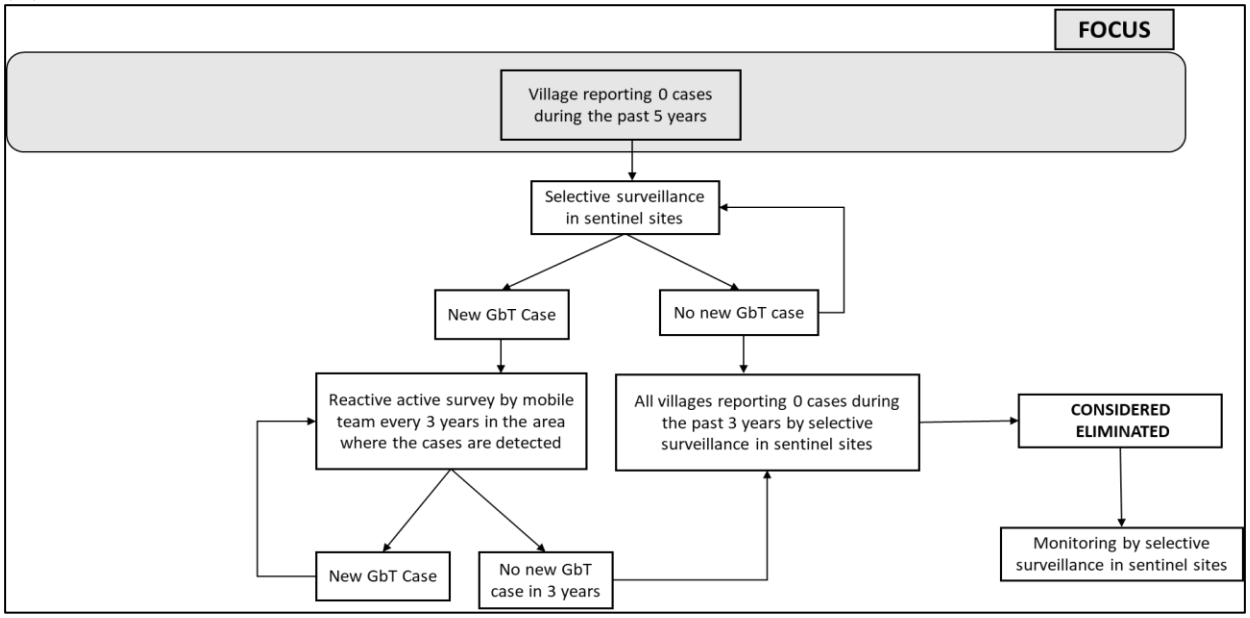


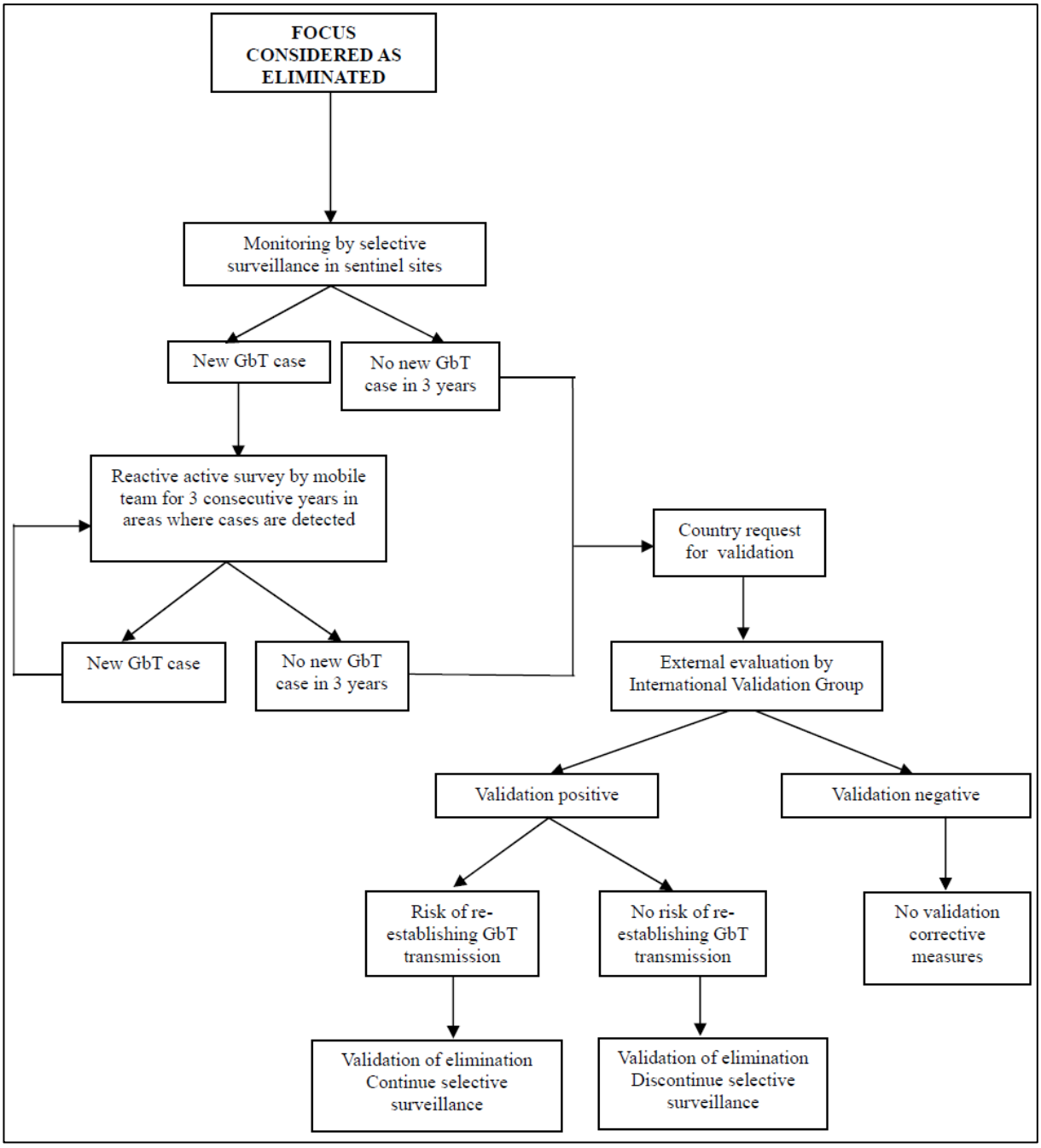
1.3 Figure 3. Strategy for eliminating Gambiense trypanosomiasis (gHAT) (78)

(A): Strategy for eliminating Gambiense trypanosomiasis (gHAT) in foci with transmission of high and very high intensity (foci reporting on average at least 1 new case per 1000 inhabitants per year during the past 5 years).

(B): Strategy for eliminating Gambiense trypanosomiasis (gHAT) in foci with transmission of moderate intensity (foci reporting on average at least 1 new case per 10 000 inhabitants but less than 1 new case per 1000 inhabitants per year during the past 5 years).

(C): Strategy for eliminating Gambiense trypanosomiasis (gHAT) in foci with transmission of low and very low intensity (foci reporting on average at least 1 new case per 1 000 000 but less than 1 new case per 10 000 inhabitants per year during the past 5 years).

(D): Strategy for eliminating gHAT in foci reporting zero cases during the past 5 years



1.4 Figure 4. Strategy for monitoring and evaluating a focus of Gambiense trypanosomiasis (gHAT) declared eliminated and process of validation (78)

2.4. gHAT trend and people at risk

The epidemiological trend of gHAT can be divided into 3 periods of time: the pre-colonial period, the colonial period and the post-colonial period.

The precolonial and colonial period

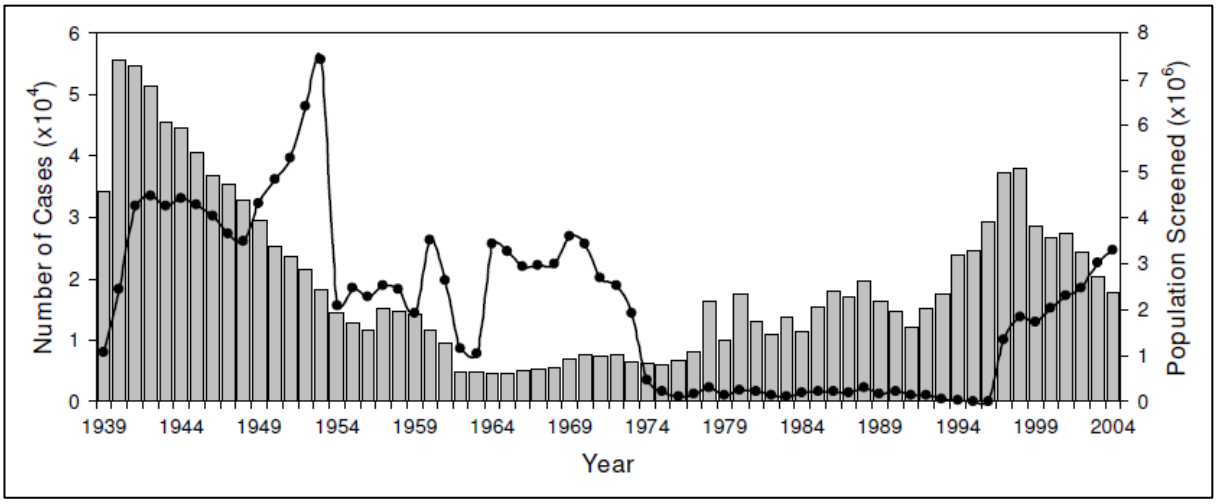
In fact, major epidemics have devastated West and Central Africa and caused number of deaths in the past, preceding the disease resurgence in the 1920s. The outbreak for this period lasted for at least one decade from 1920s – 1930s, a period of economic and social development, characterized by increased population mobility, which facilitated the spread of infection. To overcome this outbreak, colonial governments responded by implementing active approach where mobile teams were going through villages to conduct systematic population screening and treatment of cases, complemented by vector control activities. This active screening and treatment strategy elaborated by Jamot was successful, and, although it required a sustained effort over decades, this approach reduced the annual number of cases reported from 30,000 in 1920s – 1930s, to near elimination, with around 4000 cases in Africa by 1960 (3), when most of African countries got independence.

The post-colonial period

Following the independence, after a decade of low endemicity, the control of trypanosomiasis was no longer a priority and therefore, control programmes were stopped, and population screening declined to very small numbers of people (Fig 5) (83). The surveillance of gHAT decreased and was curtailed in many areas due to lack of resources and attention by the newly independent countries public health authorities. In addition, conflicts, insecurity, political turmoil, and internationalized civil war constrained disease control interventions. Since the mid-1970s there was a steady increase in the number of reported sleeping sickness to reach in the 1990s the levels of the epidemics seen at the beginning of the century, with reported annual number of cases of gHAT in 1995 – 1998 comparable to the 1920s – 1930s outbreak (Fig 5) (83). This brought country to create gHAT control programmes to fight the disease.

WHO the international partners, NGO, pharmaceutical compagnies, research organizations etc. to responded to the new epidemic gaining political will and raising new resources from public and private sectors to improve control. This resulted in a substantial reduction in the annual number of cases reported, which dropped from 37,385 in 1998 (the pic of outbreak) (81) to 11,372, in 2006, as 69.6% reduction. At the same time the number of people screened passively increased with the number of health care facilities involved in screening and the performance of active case-finding surveys improved (18). Therefore, based on those encouraging achievements, the disease epidemiological knowledge acquired, and the political will expressed by the Heads of State of endemic countries to eradicate the tsetse fly and the trypanosomes (Lomé, 2000), the WHO informal consultation of the heads of national sleeping sickness control programs (NSSCPs) held in Geneva, in May

2007, reached the conclusion that elimination of gHAT as a public health problem (PHP) was possible by 2020 (78, 106). To assist countries in monitoring the process towards this objective, many activities were planning such as the Atlas of human African trypanosomiasis, initiated in 2007 by WHO in collaboration with the Food and Agriculture Organization of the United Nations (FAO) in the framework of the programme against African trypanosomosis (PAAT). The HAT Atlas is a tool using spatial analytical methods and that allows spatial presentation and analysis of HAT burden from HAT cases data and location, monitoring of changes in distribution and epidemiology of the disease, and estimation of the size and location of populations at risk. The Atlas of HAT is in fact a useful tool for HAT control and research, whose maps and background database can be used by programmes to plan and monitor control activities and to identify epidemiological trends. And where HAT maps combined with other geospatial datasets may provide new insights on HAT distribution in relation to livestock populations, vegetation and tsetse distribution (3).



1.5 Figure 5. Number of reported cases of sleeping sickness and population screened, 1939–2004. Grey columns, number of reported cases; black circles, population screened (83).

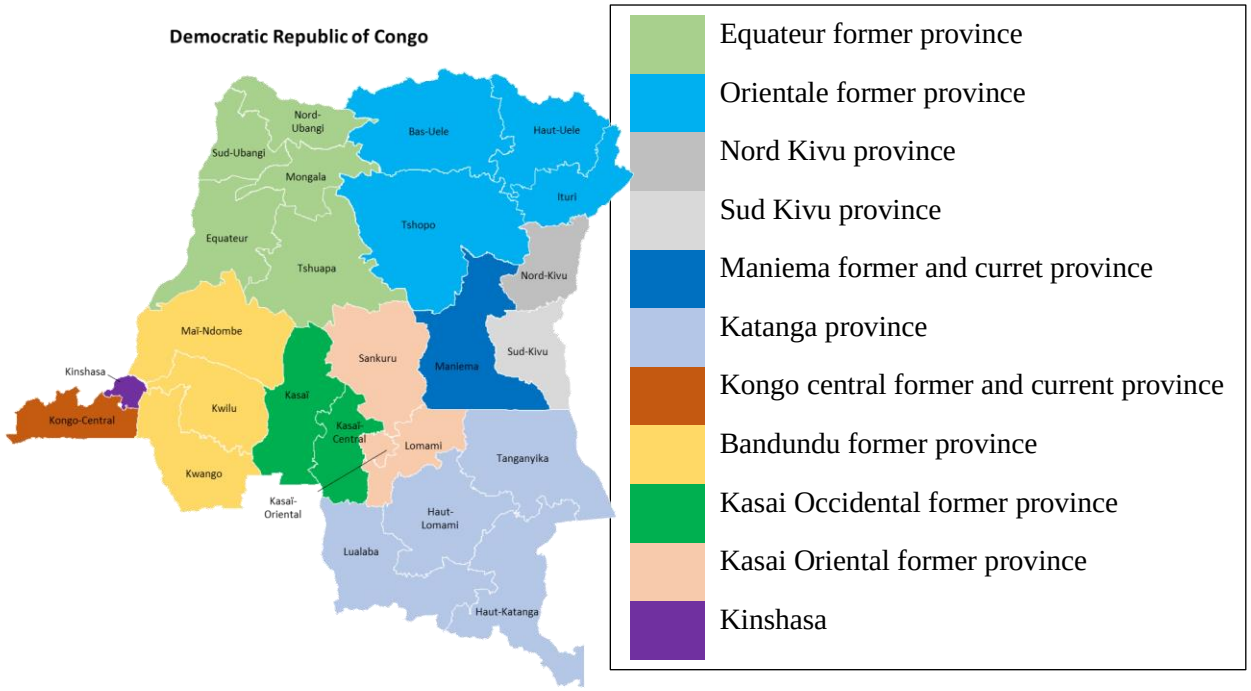
3. gHAT trend and control in DRC towards elimination

3.1. Background

Health system in DRC is organized in three levels: (a) the national (central) level with normative and regulatory role, (b) the intermediate provincial level, acting as technical support to the health districts, in charge to implement the norms from the central level and translate it in operational terms for the lower level, and (c) the peripheral and operational level of health district (HD) (health zone (Zone de Santé, ZS is short) in DRC), in charge of the implementation of the national health policy which is based on primary health care (107). Since 2015, DRC has been subdivided from 11 former provinces into 26 provinces (Fig. 6); each province being subdivided into HD from health management perspective (108, 109). Each HD account for around 100,000

inhabitants in rural region and around 150,000 inhabitants in urban regions. It's subdivided into health areas (aire de santé in DRC), and each health area has 5,000 to 10,000 inhabitants within a radius of 5-8 km, equivalent to a one-hour walk. In 2017, DRC accounted 519 HDs (S1 Data. Population per Health district in 2014 and 2017).

The national level account the public health minister and its cabinet, the general secretary with different directorates including the one in charge of disease control, the General Directorate for Disease Control (Direction Générale de Lutte contre la Maladie, DGLM in short). The DGLM coordinates three directorates in charge of different components regarding disease control, including the epidemiological surveillance directorate (Direction de Surveillance épidémiologique, DSE in short), the Health Laboratories Directorate (Direction des Laboratoires de la Santé, DLS in short), and the public hygiene and sanitation directorate (Direction de l'Hygiène et Salubrité Publique, DHSP in short), and different disease control programs.

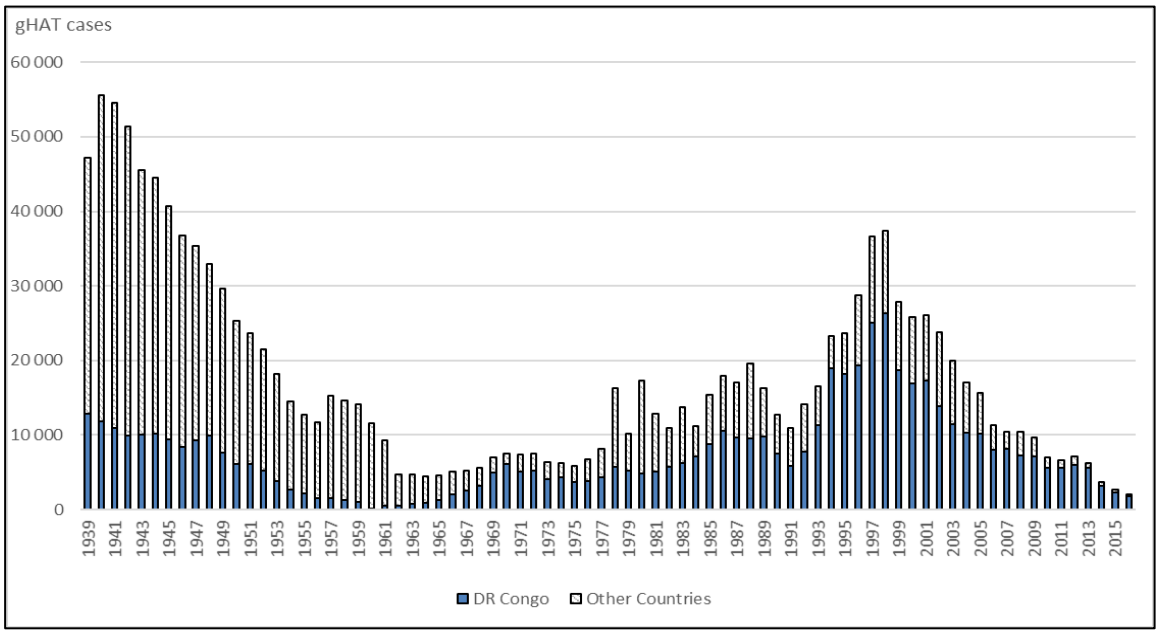


1.6 Figure 6. Democratic Republic of Congo, former and current provinces

3.2. gHAT in DRC

DRC is the most affected gHAT country, that reporting from 50 to 90% of all gHAT cases from 1990s (Fig 7). From 1998 till 2018, DRC reported 187,689 gHAT cases, equivalent to 68.7% of all gHAT cases for the same period (3, 4, 80).

DRC have a NSSCP, the "*Programme National de Lutte contre la Trypanosomiase Humaine Africaine*" (PNLTHA in short) in charge of gHAT control and surveillance activities countrywide. This programme was created in 1967, while the disease was re-emerging following the decrease in incidence after the independence of DRC. In 2017, PNLTHA was organized in 11 gHAT control provincial coordination, 29 truck based mobile teams and 27 gHAT specialized health centers, 697 PHC facilities (S2 Data: HAT Passive Screening Implementation in 2017 in DRC).



1.7 Figure 7. gHAT in DR Congo and other affected countries from 1939 to 2016. Data derived from (110, 111)

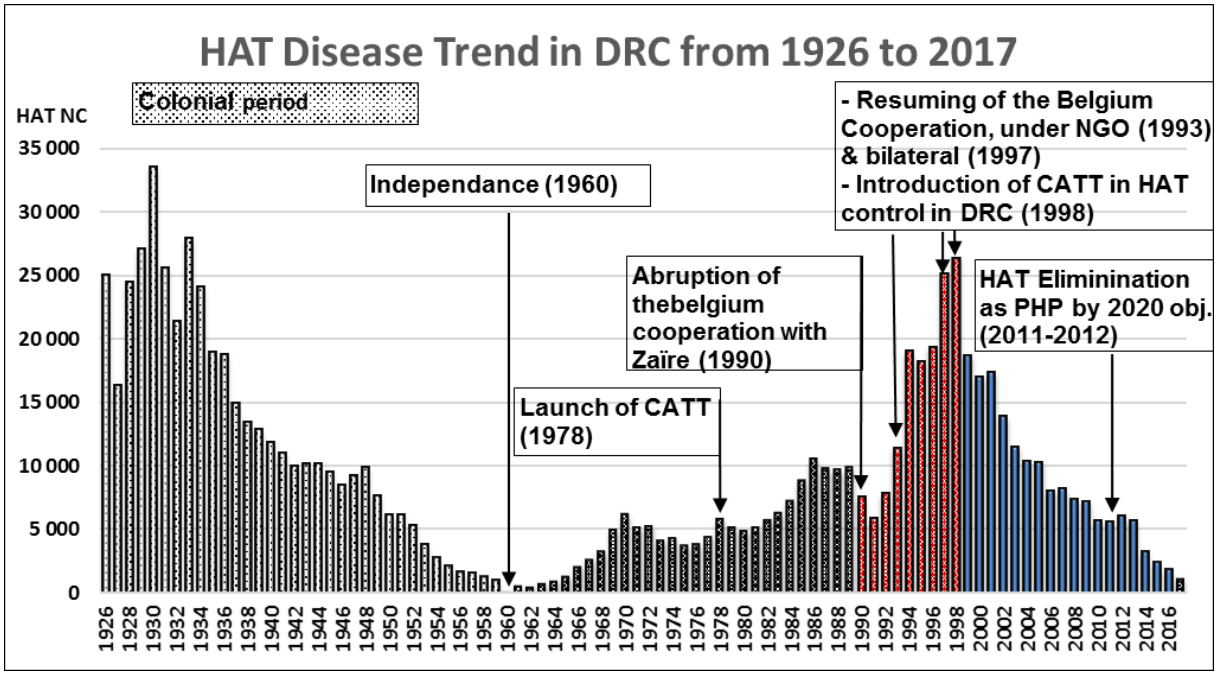
3.3. gHAT epidemiological trend in DRC from 1900s.

The epidemiological trend of gHAT in DRC from 1920s (Figs 7 & 8) is comparable and superposable to that of the whole Africa (112, 113):

- The outbreak in the 1920s – 1930s followed by the elimination of gHAT as PHP at the eve of independence of DRC in 1960 (colonial period);
- The re-emergence of disease during 1970s till the outbreak in 1990s comparable to the one of 1920s-1930s, following the breakdown of bilateral cooperation between Belgium and the republic of Zaïre (DRC) in 1990, that ended in the outbreak pic in 1998 with the resume of the cooperation with international Non-Governmental Organization (NGO) (multilateral cooperation, from year 1993) and

with Belgium (bilateral cooperation, from year 1997). In fact, Belgium Kingdom was the main sponsor financing gHAT control activities, especially the active screening by mobile teams;

- A steady declining in gHAT burden from 1998 till nowadays, supported by uninterrupted control activities with the support of different partners.



1.8 Figure 8. Trend of number of gHAT cases since 1926 till 2017in DRC

The elimination of gHAT as PHP at eve of independence (1960) was achieved when diagnostic tests were rudimentary compared to those used today. However, the screening coverage of the population at-risk and the attendance rate to active screening by mobile teams were close to 100%. Indeed, gHAT screening was mandatory and coercive during the colonial period under risk of penalties and sanctions (84). Unfortunately, during the decade following the independence of DRC, the disease resurgence happened. In fact, following the independence, the country was not able to mobilize sufficient resources to implement active screening as performed previously. With limited resources the country opted for passive screening as main strategy, integrating gHAT activities in a weak and poorly funded health system, ending in disease resurgence. To cope with this gHAT resurgence, the country created NSSCP, the BCT (Bureau Central de la Trypanosomiase) (1967), under the direct supervision of the presidency of the republic of Zaïre, before it passes under supervision of the public health ministry (2003) as PNLTHA. The BCT was mainly supported by the Belgian technical cooperation (BTC) NGO through bilateral cooperation system. In 1990 year, due to political disagreement between the kingdom of Belgium and the republic of Zaire, the bilateral cooperation between

two countries was disrupted. With that, the support to gHAT activities was stopped especially the active screening by mobile teams. The cooperation with Belgium was resumed through support of NGO in 1993 and bilateral in 1997 year. In addition to that CATT was introduced in 1996 year. Thanks to all this, the burden of the disease accumulated during almost a decade came up and revealed in 1998 years an outbreak comparable to the one in colonial period in 1920s – 1930s. From 2000s, thanks to the resumed bilateral cooperation with Belgium, the support of WHO and different other sponsors and stakeholders involved, thanks to intensification of control efforts, the steep increase in resource allocation, drug availability, the systematic use of CATT as the serologic screening test contributed to a decline in transmission gHAT burden (113). The number of cases decreased from 26318 in 1998 to 2110 (92.0% decrease) in 2016 and 953 in 2018 (96.4% decrease) marking a serious step towards the elimination of gHAT in DRC.

Analysis of the trend of gHAT in the DRC from colonial period till 2000 years reveals the following factors as determinants of the disease control:

i. Active screening of the population at risk; every time this one was stopped (decade post-independence, decade post-abruption of Belgium cooperation), the disease resurged.
ii. Coverage for the disease, as proportion of at-risk people screened
iii. Diagnostic and therapeutic tools: CATT, developed in 1978, introduced as gHAT screening test in the DRC in 1996, and drugs like DFMO, NECT; while melarsoprol faced resistance in some province like Kasai Oriental and Equateur and appeared as limitation to screening attendance among people.

Now that the evolution of the disease seems inexorable towards the elimination of gHAT in the DRC, considering the fact that this disease trend observed at the national level, is certainly not the same at the subnational level (84), we aim to evaluate the disease trend from the national level to the subnational level as well as the quality and coverage of gHAT control activities between 1998 and 2016.

3.4. gHAT control and surveillance activities and achievement analysis approach

We used gHAT data collected/archived in the WHO Atlas of HAT (available from 2000) to assess the disease trend until 2016 at country, provincial and health district levels.

We assessed the achievement regarding gHAT elimination as PHP indicators at country level during the period of 5 years from 2012 (the year when the objective to eliminate gHAT as PHP by 2020 was adopted) compared to 2000 – 2004 period-time, according to Franco *et al* (2020) (4). We assessed the intensity of gHAT transmission per health district through the yearly average gHAT case number among 10,000 inhabitants from 2000 to 2004 and from 2012 to 2016, using the population reported by HDs in 2014 (for the 2012 – 2016 period), and from this one we estimated the population of 2002 to assess the transmission intensity for the period from 2000 to 2004, using the population growth rate of 3%.

Regarding intensity and effectiveness of gHAT control and surveillance activities, we assessed the compliance of active screening implementation with the algorithms as recommended according to HAT focus transmission intensity (Figs 3) (78) during the 5 years, as well as the at-risk people passive screening coverage in 2017 at provincial and health district levels. Regarding passive screening, each health area where at least one gHAT case has been reported during the last 5 years, considered as gHAT endemic health area, have to be covered by at least a screening health facility. A health district that reports at least 1 gHAT case (endemic health district) must be covered by a referential health facility implementing gHAT screening, confirmatory and all stages case management (full package). We considered the 5 years period from 2012 – 2016 to assess the passive screening coverage in 2017 according to data base provided by DRC PNLTHA and archived by WHO (Atlas of HAT)

To assess the level of compliance with the algorithm to identify and implement active screening (AS) in the villages (Figs 3) on the 5 years period from 2012 to 2016, we used HAT Atlas data from 2007 to 2016 and estimated

- the proportion of villages targeted for active screening (AS) according to the recommended AS implementation algorithms and visited by a mobile team yearly and over the 5-year period from 2012 to 2016, among all villages visited by a mobile team
- the proportion of villages not targeted for AS according to the recommended AS implementation algorithms, that were visited by a mobile team, and those among them that reported gHAT cases among all villages that reported gHAT cases during the period (2012-2016).

This assessment was carried out in 5 provinces sampled based on the comparison of the total number of gHAT cases from 2000 to 2016 with the total gHAT cases from 2012 to 2016; 2 provinces remained highly affected compared with the others, 2 provinces where a remarkable decrease occurred, and 1 province where the decrease was minimal compared with the others.

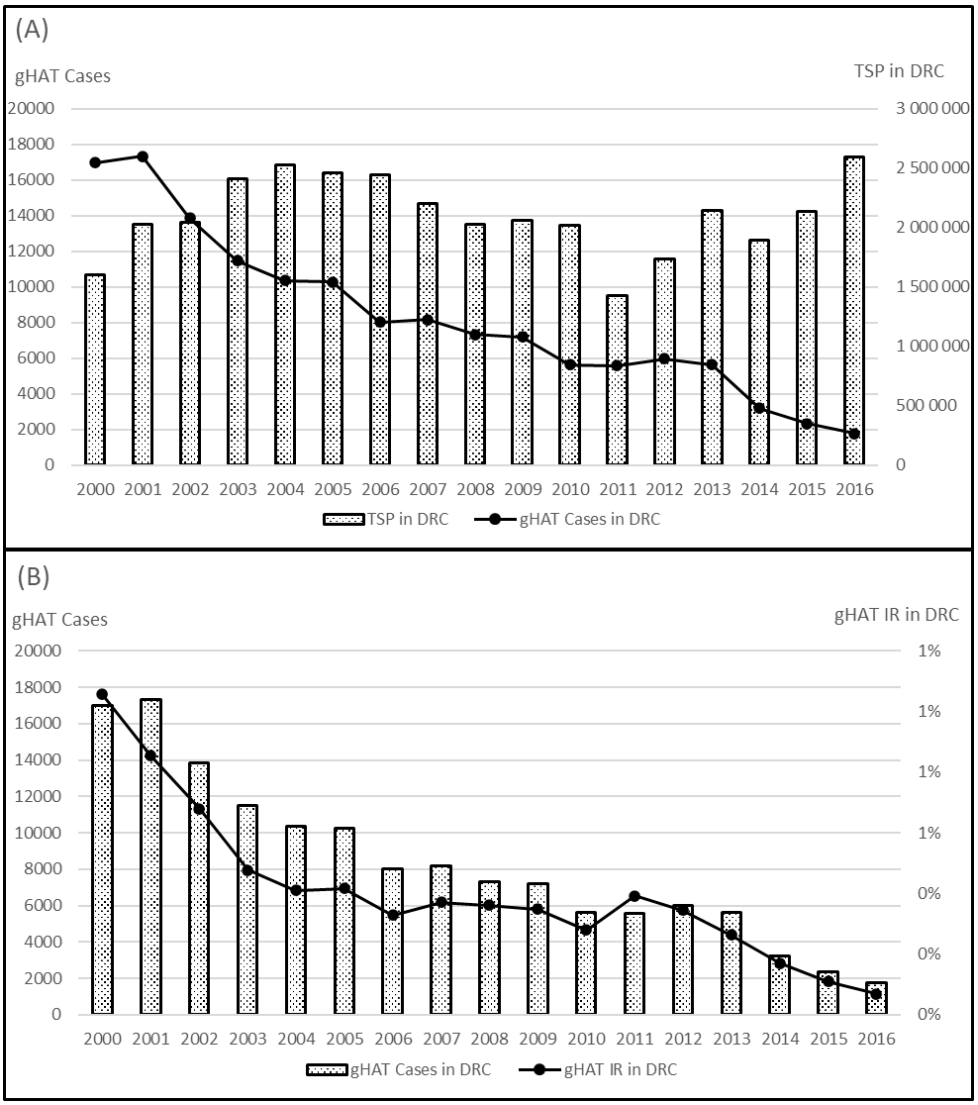
To assess passive screening coverage, we evaluated the following parameters in 2017:

- The proportion of endemic Health Districts (EHD) with at least one health facility implementing the full package of gHAT control activities;
- The proportion of endemic Health Areas (EHA) with at least one health facility implementing gHAT serological screening;
- The proportion of EHA populations within 5-8 km of a healthcare facility performing gHAT serological screening tests.

3.5. gHAT epidemiological trend in DRC from 2000 to 2016

gHAT epidemiological trend at country level

From 2000 to 2016, the number of gHAT cases in DRC decreased from 16,951 to 1,768 as 89.6% of decrease. This decreasing has been gradual, punctuated by stagnations every 2-4 years, with 2000-2001, 2004-2005, 2006-2009 and 2010-2013 plateaus (Fig. 9). As the number of detected cases is correlated to the gHAT total screened people number (TSP), that reflects the screening coverage, we analyzed the trend of the number of cases in relation to the TSP between from 2000 and 2016 and the extent of the disease reflected by the HAT Annual Detection Rate (ADR) (proportion of patients detected among all those screened) or Infection Rate (IR). The fact that the number of gHAT is decreasing while the TSP is maintained or increasing concomitantly to a decreasing ADR confirm that the extend (burden) of the disease is really decreasing. The sudden increase



1.9 Figure 9. gHAT trend in DRC from 2000 till 2016

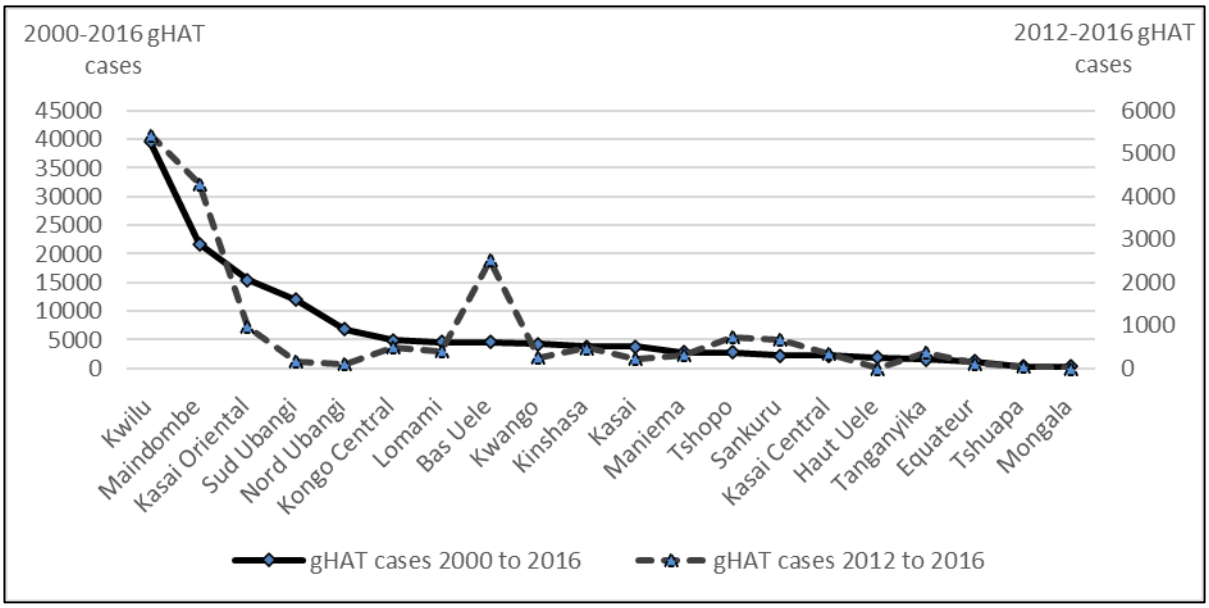
of gHAT ADR that happened in 2011 – 2013 is concomitant to decrease of TSP and increase / stagnation of number of gHAT cases reported during the period (Fig 9).

gHAT epidemiological trend at provincial level

Kwilu and Maindombe provinces, (parts of the former Bandundu province) reported the highest number of gHAT cases between 2000 to 2016, followed by Kasai Oriental, Sud Ubangi and Nord Ubangi (parts of the former Equateur province), Kongo Central and so on (Bas-Uele, Lomami and Kwango). The provinces of Haut Lomami, Sud Kivu, Lualaba, Haut Katanga, Nord Kivu and Ituri that reported average 0-2 cases of HAT in a year are classified as non-endemic, as the reported cases were obviously imported cases infected in one of the surrounding endemic provinces. Thus, 20 of the 26 provinces in the DRC are classified as gHAT endemic for the period from 2000 to 2016. (S3 Data. gHAT cases in DRC from 2000 to 2016).

To assess changes in gHAT endemicity between 2000 and 2016, we compared results from 2000 to 2016 with those from 2012 to 2016. Kwilu and Maindombe provinces remained the most prevalent for gHAT based on the total number of cases reported over the two time periods, followed by Kasai Oriental and Kongo Central provinces. Nord Ubangi and Sud Ubangi provinces, among the most prevalent over the entire 2000-2016 period, are among the least endemic of all over the 2012-2016 period. (Fig. 10).

Eight of the 20 HAT endemic provinces experienced a decrease in cases between 2000 and 2016 equal to or greater than the national average (89.6%): Nord Ubangi and Sud Ubangi (decrease of at least 99%), Kasai Oriental, Kasaï, Equateur, Mongala, Haut Uélé and Bas Uélé (the last 3 did not report a single case in 2016 and thus experienced a 100% decrease). The provinces of Kasai Central, Kwilu, Maniema and Maindombe, Sankuru and Tanganyika experienced a lower proportion of case reduction between 2000 and 2016 compared to the national average (S3 Data. gHAT cases in DRC from 2000 to 2016).



1.10 Figure 10. gHAT cases detected per province from 2000 to 2016

gHAT disease trend analysis at the Health District level

From 2000 to 2016, 266 Health Districts (HDs) reported at least one case of gHAT including nine of these located in the provinces of Bas Uele (Ango, Bondo, Ganga, Poko and Titule HDs) and Haut Uele (Doruma, Dungu, Niangara and Rungu HDs) that reported 4.7% of total gHAT cases. These nine HDs were covered by MSF, they reported most of cases (over 90%) between 2007 and 2013 years. Our analysis will focus on HDs covered by the PNLTHA outside of these 9 HDs, as policies and case definitions used by MSF were different from the one by PNLTHA, the DRC NSSCP. Among the 257 HDs covered by PNLTHA, that reported at least one gHAT case from 2000 to 2016, 204 (79.4%) remain endemic, reporting at least one case from 2012 to 2016. The remaining 53 (20,6%) accounted for less than 1% (0,8%) of gHAT cases reported from 2000 to 2016. (S1 Data gHAT cases in DRC from 2000 to 2016).

Twenty-six of the 257 EHDs (10%) reported 60% of the cases detected from 2000 to 2016: (i) 10 in Kwilu province (Yasa Bonga, Masi Manimba, Bandundu, Kikongo, Bulungu, Kimputu, Djuma, Bagata, Mokala, Ipamu), (ii) 6 in Maindombe province (Kwamouth, Bolobo, Nioki, Bokoro, Mushie, Yumbi), (iii) 2 in Kasai Oriental province (Bibanga, Mukumbi), (iv) 2 in Sud Ubangi province (Gemena, Kungu), (v) 2 in Nord Ubangi province (Karawa, Loko), (vi) 1 in Lomami province (Kalambayi Kabanga), (vii) 1 in Kwango province (Kenge), (viii) 1 in Kasai province (Kakenge), (ix) 1 in Tshopo province (Isangi). Nineteen of these 257 HDs (7,4%) among the 26 HDs (in Kwilu, Maindombe, Kwango, Tshopo and Kasai Oriental provinces) reported 62,8% of all cases detected from 2012 to 2016.

From 2000 to 2016 the number of cases reported in the DRC decreased of near 90% while the number of HDs notifying gHAT cases changed little, decreasing just of 20,6%.

3.6. Assessment of gHAT intensity of transmission per health district

From 2000 to 2004, 225 HDs reported at least one gHAT case (S1 Data. gHAT cases in DRC from 2000 to 2016), 113 at very low or low transmission intensity (<1 gHAT / 10,000 inhabitant), 78 at moderate transmission intensity (≥1 gHAT / 10,000 inhabitant & <1 gHAT / 1,000 inhabitant) and 34 at high or very high transmission intensity (≥1 gHAT / 1,000 inhabitant). The 78 HDs at moderate transmission intensity were distributed among all 18 endemic provinces (out of Bas Uele and Haut Uele) and the 34 HDs at high and very high transmission intensity were distributed among 8 of the 18 provinces (Table 3).

From 2012 to 2016, among the 204 endemic HDs that reported at least one cased, 163 were at very low or low transmission intensity, 39 at moderate transmission intensity and 2 at high or very high transmission intensity (Bolobo and Kwamouth HDs, both in Maindombe province). The 39 HDs at moderate transmission intensity were distributed in 13 provinces (Table 3), 19 of these were at high or very high transmission intensity for the 2000 - 2004 period, 17 at moderate level, and 3 at low or very low transmission intensity. The 2 HDs at high or very high transmission intensity during 2012-2016 period were at the same level for 2000 – 2004 period (S4 Data. Assessment of gHAT intensity of transmission per health district in DRC).

1.3. Table 3: Number of EHD* per gHAT transmission intensity per province

Province	2000-2004				2012-2016			
	Zero case	Low & very low	Moderate	High & very high	Zero case	Low & very low	Moderate	High & very high
Equateur	3	8	2	0	6	7	0	0
Kasai	3	4	3	1	3	8	0	0
Kasai Central	8	10	4	0	3	18	1	0
Kasai Oriental	0	2	13	4	0	17	2	0
Kinshasa	1	29	5	0	10	24	1	0
Kongo Central	1	15	9	2	8	16	3	0
Kwango	1	2	2	2	2	4	1	0
Kwilu	3	5	6	10	4	6	14	0
Lomami	1	8	5	0	1	12	1	0
Maindombe	0	5	3	6	1	5	6	2
Maniema	0	5	5	0	1	8	1	0
Mongala	2	2	1	0	4	1	0	0
Nord Ubangi	0	2	3	2	2	5	0	0
Sankuru	3	5	4	0	2	6	4	0
Sud Ubangi	0	1	8	7	3	12	1	0
Tanganyika	0	5	2	0	2	3	2	0
Tshopo	5	4	2	0	1	8	2	0
Tshuapa	1	1	1	0	0	3	0	0
Total	**32**	**113**	**78**	**34**	**53**	**163**	**39**	**2**

*Only HDs that reported at least one case from 2000 to 2016 are concerned

3.7. gHAT active screening implementation assessment from 2012 to 2016

According to the different algorithms to implement active screening, a village that has reported at least 1 gHAT case in the past 3 years is targeted for active screening once a year for the next 3 years; a village that has reported at least 1 gHAT case in the last 5 years and no case during the past 3 years is targeted for active screening every 3 years until no cases are detected for 5 years (at which point the village is no longer targeted for active screening). In low and very low transmission intensity foci, village where no gHAT case are detected in 3 successive years are no longer targeted for active screening. Our assessment thus focused on a 3-year period. We assessed the compliance with the active screening implementation algorithms in the 5 provinces of Maindombe, Kwilu, Kongo Central, Sud Ubangi and Kasai Oriental.

A total of 2539 villages were eligible for active screening each year from 2012 to 2014, 315 of these (12.4%) were visited by a mobile team and subjected to active screening each year during the period; the proportion of eligible villages visited at the level of each of these provinces have been 18.6% for Maindombe province, 10-20% for Kongo Central, Kasai Oriental, and Kwilu, and 1.4% for Sud Ubangi province. Among these villages targeted for active screening yearly between 2012 and 2014, 60.5% were visited by a mobile team at least once over the 3 years (ranging from 52.3%, for Sud Ubangi, to 62.9% for Kwilu province) and 34.3% were visited at least twice (ranging from 17.6% for Sud Ubangi, to 37.7% for Maindombe province) during the period.

A proportion of 26.9% (744) of the villages visited by a mobile team even once during the 2012 – 2014 period were not listed as eligible for active screening at all (ranging from 22.9% for Kasai Oriental, to 38.5% for Sud Ubangi) and only 10.2% (76) of these villages reported cases (ranging from 4.0% for Sud Ubangi and Kasai Oriental, to 15.6% for Maindombe). However, 53.2% (1075) of the villages targeted for active screening even once during the 2012 – 2014 period and that were actually visited by a mobile team even once reported HAT cases (ranging from 17.7% Sud Ubangi to 71.7% for Maindombe) (Table 4 & S3 Data: Active Screening assessment 2012-2016)

1.4. Table 4: Number of villages eligible and subjected to active screening from 2012 to 2014

Province	Eligible to AS yearly (=A)	Visited by MT yearly among " A"	Visited at least one year among " A"	Visited at least two years among " A"	Visited at least once, non-eligible (=B)	Eligible to AS at least one year & visited at least one year among	Villages that reported HAT case	Villages with HAT cases among " B"
Kasaï Oriental	347	47	208	121	99	333	184	4
Maindombe	462	86	281	174	135	371	287	21
Kwilu	1308	143	823	475	325	1004	578	42
Kongo Central	206	36	110	64	86	155	70	5
Sud Ubangi	216	3	113	38	99	158	32	4
Total	**2539**	**315**	**1535**	**872**	**744**	**2021**	**1151**	**76**

Considering the 3-year period from 2014 to 2016, 1913 villages were to be visited by a mobile team (eligible for active screening) each year. Only 20.0% (382) of these villages were subjected to active screening yearly (5.6% for Kongo Central, 36.8% for Maindombe and proportion ranging between 14 and 20% for the 3 other provinces), 63.7% (1219) were subjected to active screening at least once (ranging from 48.4%, for Kongo Central, to 82.5% for Maindombe) and 42.6% (815) at least twice (ranging from 19.9% for Kongo Central, to 62.3% for Maindombe province) over the 3 years.

Among all villages visited by a mobile team at least once during the period, 33.2% (966) were not eligible for active even once during the 2014 – 2016 period (ranging from 24.7% for Sud Ubangi, to 36.1% for

Maindombe) and 6.8% (66) of these (ranging from 0.0% for Sud Ubangi, to 36.1% for Kasai Oriental) reported HAT case while 40.8% (794) of the villages eligible for and subjected to active screening at least once during the 2014 – 2016 period, reported at least one gHAT case (ranging from 15.2% Sud Ubangi to 63.5% for Maindombe) (Table 5 & S3 Data Active screening assessment 2012-2016)

1.5. Table 5: Number of villages eligible and subjected to active screening from 2014 to 2016

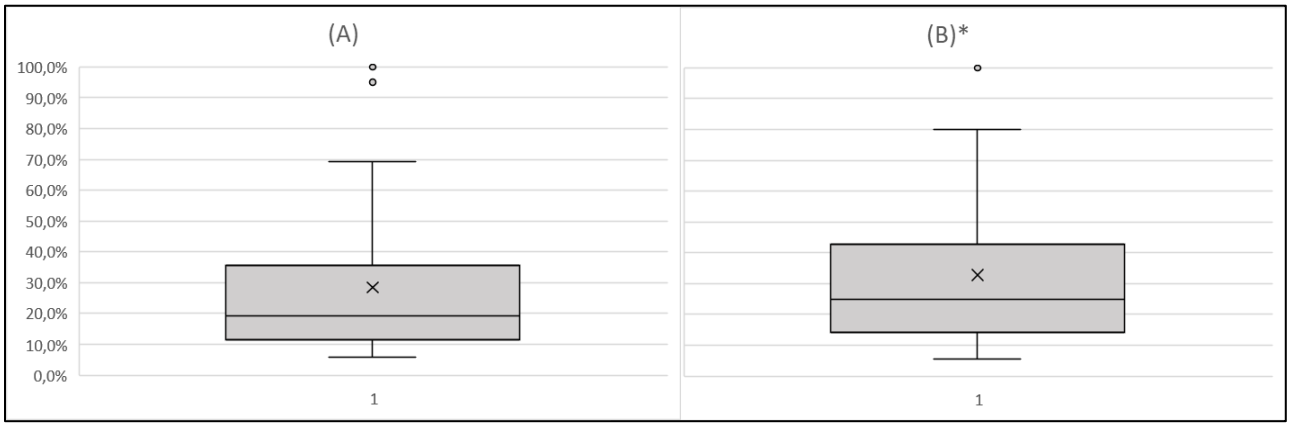
Province	Eligible to AS yearly (=A)	Visited by MT yearly among " A"	Visited at least one year among " A"	Visited at least two years among " A"	Visited at least once, non-eligible (=B)	Eligible to AS at least one year & visited at least one year among	Villages that reported HAT case	Villages with HAT cases among " B"
Kasaï Oriental	301	52	176	102	120	297	99	13
Maindombe	326	120	269	203	247	438	299	21
Kwilu	977	180	606	420	496	961	382	27
Kongo Central	161	9	78	32	62	123	61	5
Sud Ubangi	148	21	90	58	41	125	19	0
Total	**1913**	**382**	**1219**	**815**	**966**	**1944**	**860**	**66**

3.8. gHAT passive screening coverage

Among the 41 HDs with moderate, high, or very high transmission intensity of gHAT transmission for the 2012-2016 period, 38 (92.7%), excluding the HDs of Boma Bungu (Kongo Central province), Boto (Sud Ubangi province) and Mbulula (Tanganyika province), were covered by at least one referral health facility implementing the full package of gHAT activities (screening, diagnostic, staging and treating of 1st and 2nd stage patients). Of the 163 low- and very low- transmission intensity HDs, 56 (34.4%) were covered by a full package of gHAT activities (S2 Data. gHAT passive detection and management coverage in 2017).

At the health area (HA) level, the proportion of HAs that reported at least one gHAT case from 2012 to 2016 covered by a health facility that was implementing serological screening was 32.0% in Bolobo and 40.9% in Kwamouth, the 2 high and very high transmission intensity HDs. For the 39 HDs with moderate transmission intensity, the proportion of HA with a health facility implementing serological screening ranged from 0.0% (for Mbulula HD, in Tanganyika province and Lubunga HD in Kasai province) to 100.0% (for Inga HD in Kongo Central province and Mosango HD in Kwilu province, followed by Yasa Bonga (95.0%) in Kwilu province). The median proportion was, 18.5%, with 1st quartile of 10.0%, 3rd quartile of 35.0% and 90th percentile of 58.8%. Among the 163 low- and very low-intensity transmission HDs, those that achieved the elimination of gHAT as PHP threshold, in 89 HDs (54.6%), no health facility (HF) was performing serological screening. In the remaining 74 HDs, the proportion of HA covered by a gHAT serological screening HF per HD ranged from 5.6% (Masuika HD in Kasai Central province) to 100.0% (Muetshi HD, in Kasai Central,

Kingabua HD in Kinshasa, Bena Dibele HD, in Sankuru HD, Mangembo and Tshela HDs in Kongo Central province) with median at 25.0%, 1st quartile at 14.3 %, 3rd quartile at 42.1% and 90th percentile at 71.4%. (Fig 11) Data on proportion of HAs covered by a health facility implementing screening are provided in S3 Data



1.11 Figure 11. Proportion of HA with a gHAT serological screening HF among HD with very high, high & moderate (A) and with low and very low (B) transmission intensity
*the zero case HD are not considered here

We assessed the proportion of people within 5 to 8 Kms of a health facility implementing gHAT control activities (screening, diagnostic, treatment for 1st and 2nd gHAT stages) (S5 Data). Around 21.4% of the population was located within 5 km of a health facility implementing gHAT serological screening and 56.3% within 8 km in the Bolobo HD; and 7.4% within 5 km and 15.9% within 8 km in the Kwamouth HD, the two HDs with high or very high intensity of gHAT transmission for the 2012 – 2016 period. For the 39 HDs at moderate level of gHAT transmission intensity, the proportion of people within 5 Kms of a gHAT serological screening health facility ranged from 0.0% (for Mbulula HD, in Tanganyika province and Lubunga HD in Kasai province) to 83.4% (for Bandundu HD in Kwilu province). The median proportion was 16.5%, with 1st quartile of 7.2%, 3rd quartile of 32.9% and 90th percentile of 42.6%. Over a wider radius, the proportion of people within 8 Kms of a gHAT serological screening health facility ranged from 0.0% (for Mbulula HD, in Tanganyika province and Lubunga HD in Kasai province) to 92.7% (for Yasa Bonga HD in Kwilu province, immediately followed by Bandundu HD with 90.2%). The median proportion was 29.1%, with 1st quartile of 15.5%, 3rd quartile of 51.5% and 90th percentile of 59.4%.

Among the 163 low- and very low- transmission intensity HDs, 40 HDs (24.5%) had no one people within the 5 Kms of a gHAT serological screening health facility. For the 123 remaining HDs the proportion of people within 5 Kms of a gHAT serological screening health facility ranged from 0.1% (for Lodja HD in Sankuru province and Moanza in Kwilu province) to 100% (for number of urban HDs in Kinshasa and Kasai Oriental provinces). The median proportion was 28.2%, with 1st quartile of 3.6%, 3rd quartile of 91.0% and 90th

percentile of 100.0%. The proportion of people within 8 Kms of a gHAT serological screening health facility ranged from 0.1% (for Kimbao HD, in Kwilu province and Lubunga HD in Kasai province) to 100.0% (for number of urban areas). The median proportion was 51.1%, with 1st quartile of 7.5%, 3rd quartile of 100.0% and 90th percentile of 100.0%.

3.9. gHAT control achievement toward gHAT elimination as PHP in 2017

Comparison between the 2000 – 2004 and 2012 – 2016 periods, revealed that the number of HDs with moderate, high or very high transmission intensity, and thus, HDs that did not meet the threshold for elimination of gHAT as PHP, decreased by two thirds, although three HDs with low or very low transmission intensity in 2000 – 2004 period turned at moderate level during 2012 – 2016 period. The total number of cases reported annually decreased deeply between 2000 and 2016 (89.6%), while the total number of screened people tended to remain steady over the years. Based on data from 2012 to 2016, 84.0% (216) of the HDs that reported gHAT cases from 2000 to 2016, were at low or very low transmission intensity (24.5% of these (53 HDs) didn't report any HAT case from 2012 to 2016), having thus reached the HAT elimination threshold; evidence that DRC was already moving towards elimination of gHAT as PHP. Unfortunately, the activities underpinning this achievement were not implemented as effectively and qualitatively as planned, in line with the recommendations and guidelines. Indeed, the gHAT passive and active control activities coverage remained low and the active screening algorithms were poorly implemented. However, the results were encouraging. This need to be further explored. Either the results showing a strong decrease in disease burden were really good, or there was a hidden explosive situation. In any case, the situation seems to have been good, as the decrease in the number of cases is concomitant with a maintenance of the screened population, and there was no outbreak nor an outbreak rumor. Another alternative is that people at risk were actually really attending active screening and were detected and treated whenever time a mobile team was in the village. The real challenge is to prevent a resurgence of gHAT especially when the coverage is not sufficient, and if the regularity of active screening is not maintained, some missed case could be source of reemergence. Therefore, it will be necessary to maintain surveillance in foci where the disease has been eliminated as PHP.

Supporting Information

S1 File. Number of gHAT cases by health districts, by province et in DR Congo from 1990 to 2016.

(XLSX)

S2 File. HAT Passive Screening coverage per health district and health area in 2017 in DRC. EHA: Endemic Health Area; EHD: Endemic Health District; HAT: Human African Dictrict

(XLSX)

S3 File. Active Screening assessment from 2012 to 2016

(XLSX)

S4 File 4. 2000-2004 & 2012-2016 gHAT transmission intensity per Health District in DRC

(XLSX)

4. Rationale of the thesis

Since the 1990s many efforts, in gHAT control, led (again) to a substantial reduction in the disease burden. The resumption of the cooperation and activities, the contribution of research and support from pharmaceutical companies and different stakeholders under the coordination of WHO have contributed to reducing the disease burden. As a result, the goal of eliminating gHAT as PHP had been set for 2020 and interrupting transmission for 2030. As the disease burden and number of cases decline, active screening which was successful in controlling the disease in colonial times and nowadays to set it for elimination, is becoming less cost-effective, victim of its own success. Therefore, integration of gHAT into PHC system appears to be the main alternative, complemented by reactive screening and/or active screening by mobile team that is less costly than current truck-based team.

Based on lessons learned from past successes and failures and from research (fundamental, operational and economic research), challenges have been identified for sustainable and sustained elimination of gHAT. These challenges include (i) diagnostic and therapeutic tools and strategies suitable for PHC system and health facilities in remote areas where gHAT is most prevalent, (ii) maintaining the technical capacity and attention of health care workers, and raising public awareness, (iii) ensuring sufficient coverage of control activities, (iv) addressing the question of the animal and asymptomatic reservoir, (v) implementing effective monitoring of gHAT elimination and cost-effective surveillance system to assure a sustainable elimination and diagnose promptly any risk of reemergence of disease.

Our scientific work is mainly oriented towards analyzing current results toward gHAT elimination in DRC and improving diagnostic tools and detection/treatment strategies to address the issue of integrating gHAT control into PHC system and improving coverage of activities.

Therefore, considering too the objective of sustainable elimination towards interruption of transmission, this research project had the following questions:

- Are alternative approaches and tools more sufficient than current ones in the elimination process?
- Are current strategies and tools sufficient to reach the elimination threshold?
- Is the Individual Rapid serological test (HAT RDT) effective and useful for innovative strategies to reach the elimination threshold?
- What additional activities and efforts are potentially needed to achieve gHAT elimination?
- How can gHAT be eliminated as PHP and how can this elimination be made sustainable?

In fact, to achieve the goal of sustainable elimination, it is a necessary to put in place :

- cost-effective strategies:
    - integration of activities into the general health system
    - accurate epidemiological assessment of gHAT prevalence
- Innovative and adapted tools:
    - New diagnostic tools (Rapid Diagnostic Tests (RDT) instead of CATT at screening step…)
    - New drugs
    - Performant surveillance and monitoring system.

5. Objectives of the thesis

5.1. Main objective:

To contribute to the elimination of gHAT as PHP objective through the development of innovative and new diagnostic tools and strategies.

5.2. Specific objectives:

- To assess the implementation of current control activities alongside the prevalence and trend of gHAT at country, provincial and health district levels.
- To evaluate in term of efficiency, effectiveness and cost-effectiveness new diagnostic tools to be included in different strategies.
- To evaluate an intensified (scaled up) passive screening as an innovative strategy for gHAT surveillance and control .

6. Organization of the thesis

This thesis is organized into following parts

Chapiter1: General introduction

The general introduction describes an overview on gHAT, gHAT control strategies and activities, the goal of gHAT elimination, the trend and control of gHAT in DRC, the main affected country, towards the goal of gHAT elimination, the rationale of the thesis, the studies herein and their relevance.

Chapiter 2: General methodology

In the course of this research work, whose objective was to contribute to the goal of eliminating HAT as a public health problem in the DRC (in 2020) and interrupting its transmission (in 2030), we used a situation analysis questioning the results obtained from the activities carried out until 2016. We conducted research in order to identify innovative tools and strategies for HAT detection that would be more efficient.

We analyzed the epidemiological trends of HAT between 2000 and 2016 in the DRC in view of the objective of eliminating HAT. The availability of thermostable, easy-to-perform, individualized screening tests and more efficient screening strategies, as well as the availability of effective and safe drugs, were identified as challenges to the elimination of HAT. This research project focused on diagnostic tools and strategies, evaluating the effectiveness of different individual rapid diagnostic tests compared to CATT as well as the efficiency of new diagnostic strategies combining these individual tests and other innovative diagnostic tools.

Chapiter 3: General Results

- **Result 1.**

This part is based on data analysis to review the status of sleeping sickness in DRC between 2000 and 2012, with a focus on spatio-temporal patterns and analysis of epidemiological trends at the national and provincial levels. The main source of data for this study was the DRC National Sleeping Sickness Control Programme—NSSCP and additional data provided by the Non-Governmental Organization (NGO) MSF.

- **Result 2.**

Here are reported the results of evaluation of the performance of a prototype Rapid Diagnostic Test (RDT) made of native antigens for detection of *T.b. gambiense* in human blood (1st generation HAT RDT) in comparison to Card Agglutination Test for Trypanosomiasis (CATT) from a prospective study implemented in Angola, Democratic Republic of Congo and the Central African Republic

- **Result 3.**

This part presents the results of the evaluation of the performance of the SD BIOLINE® HAT RDT and/or CATT to detect *gambiense* in various diagnostic algorithms in the former provinces of Bandundu, Kasai Occidental and Kasai Oriental of the Democratic Republic of Congo.

- **Result 4.**

Here, we report results of a cost-effectiveness analysis of CATT and SD BIOLINE® HAT RDT to detect gHAT in different diagnostic algorithms implemented by mobile teams and fixed health facilities in the former provinces of Bandundu, Kasai Occidental and Kasai Oriental of the Democratic Republic of Congo.

- **Result 5.**

This part presents the accuracy of a 2nd generation SD BIOLINE® HAT RDT, made of recombinant antigens, for the detection of *T.b. gambiense* infection from human blood in comparison with the 1st generation SD BIOLINE® HAT RDT, produced using native antigens (SD BIOLINE HAT) and CATT in a prospective study in the former province of Bandundu, in the Democratic Republic of Congo.

- **Result 6.**

This is related to the results of performance evaluation of a RDT developed for simultaneous detection of gHAT and malaria; beyond being a simple test, it represent in itself a whole strategy of integration of gHAT and other tropical diseases, malaria for this purpose, diagnosis.

- **Result 7.**

These are results of evaluation of strategy developed using different new diagnostic tools including the RDT for HAT ,to contribute to elimination of gHAT in Kongo Central, province which was reporting a relatively low, yet steady number of cases. This strategy consists of an improved and expanded passive case detection, complemented by reactive screening.

Chapiter 4: General discussion and Conclusion

This chapter presents the general discussion, the conclusion and further perspectives for gHAT research towards sustainable elimination as PHP and interruption of transmission. The general discussion reports the contribution of this research project to the gHAT elimination process and its limitations, highlights the challenges particularly with regard to a sustainable elimination of gHAT as PHP towards interruption of transmission and prevention of reemergence; and mentions the perspectives and outstanding issues.

This section contains a number of recommendations and suggestions addressed to the various stakeholders in the fight against HAT, including sleeping sickness control programs. The objective is to ensure that the current results that predict the elimination of HAT in some areas of the DRC are indeed valid, to ensure the sustainability of these results towards the cessation of transmission and that the control tools and strategies, especially those related to diagnosis, are updated for more effectiveness and efficiency towards the goal of eliminating and stopping the transmission of HAT in the whole country

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Chapter 2

General methodology

Our research project is based on six studies whose results have been shared through seven scientific articles. The methodology of each study as reported in the different publications is summarized below.

The distribution and risk of human African trypanosomiasis in the DRC were assessed for the period 2000 to 2012. Data on active and passive HAT screening were primarily provided by the DRC NSSCP and additional data were provided by the NGO MSF, which implemented activities in insecure areas of Equateur and Orientale provinces that were difficult to access for the NSCCP. Annual reports were used to establish epidemiological trends at the national and provincial levels from 2000 to 2012, while village-level data were used to construct the geospatial database, HAT distribution mapping, and risk assessment and mapping.

To address the weaknesses and limitations of the CATT, a thermostable individual screening test, the SD BIOLINE® RDT for HAT, was developed and the performance of a prototype of this test was evaluated against the CATT. Thus, the performance of the SD BIOLINE prototype RDT for HAT was evaluated on the basis of the sensitivity and specificity of this prototype to detect *T.b. gambiense* infection through a non-inferiority study comparing this prototype to CATT in the field, in a prospective multi-centre study. Participants were recruited through active and passive screening in HAT-endemic countries, namely Angola, DRC and CAR.

Once optimised, the performance of the SD BIOLINE® HAT RDT in various diagnostic algorithms for HAT in DRC, including CATT, was evaluated. Study participants were recruited through active and passive screening in Maindombe, Kwilu and Kasai provinces using CATT and SD BIOLINE® HAT RDT performed in parallel, at screening stage, in the HAT diagnostic process. Thus the performance of the screening tests in various algorithms including HAT RDT and/or CATT as screening tests was evaluated on the basis of sensitivity, specificity, positive predictive value and negative predictive value. Using the estimated sensitivity and specificity performance of SD BIOLINE® HAT RDT and CATT, cost-effectiveness analysis was performed to inform a rational choice between different screening algorithms based on SD BIOLINE® HAT RDT and/or CATT as screening tests in DRC.

The SD BIOLINE® HAT RDT mentioned above is based on native trypanosome antigens and is therefore classified as a first generation RDT. As it is based on native antigens, the production of which is subject to certain limitations, notably because ITM Antwerp is the only producer, the production of this RDT is confronted with certain difficulties and limitations, notably a risk of stock shortage, as well as a risk of contamination due to poor laboratory handling. To overcome these risks, a 2nd generation SD BIOLINE® HAT RDT (SD BIOLINE® HAT 2.0) made of recombinant antigens was developed and its performance evaluated through a prospective study. Participants in this study were recruited through active and passive

screening using CATT, SD BIOLINE® HAT RDT (1st generation) and SD BIOLINE® HAT 2.0 (2nd generation) as screening tests in Kwilu and Maindombe provinces. The performance of the different screening tests (HAT 2.0, RDT 1.0 and CATT) considered individually and variously combined was evaluated in terms of sensitivity, specificity and accuracy during the 2 types of screening (active and passive) combined or separately.

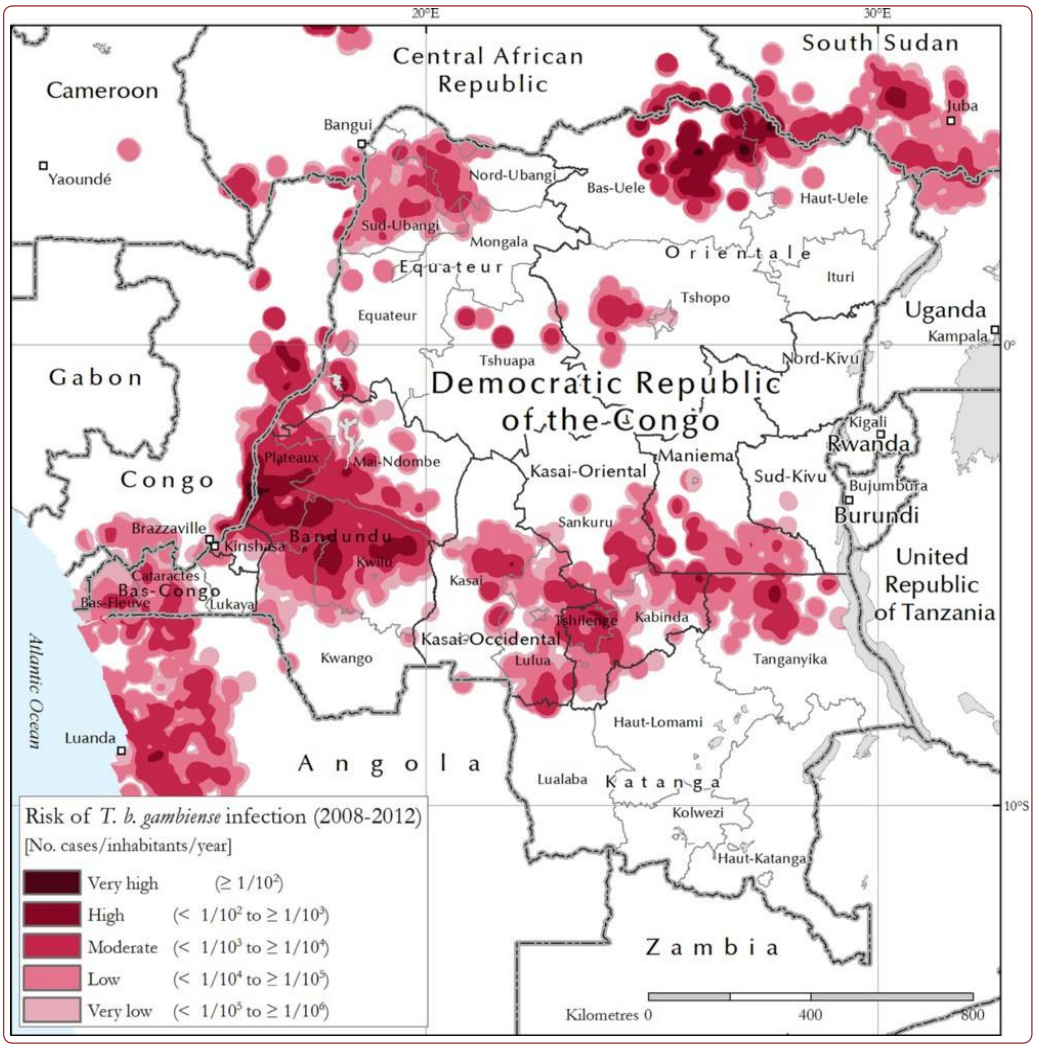
With the marked decline in the number of cases and prevalence of HAT, screening as currently implemented is becoming less cost-effective. Also, the decline in test's PPV concomitant with the decline in prevalence, which is marked for RDT, leads to an increase in false positives, which can lead to a decline in interest and confidence in test results among health workers. One solution is to integrate the diagnosis of HAT into the diagnostic process of other tropical diseases, such as malaria, whose clinical signs are similar to those of HAT. We have therefore chosen to integrate HAT screening into the malaria detection process through the development of a combined RDT. Thus, the performance of a prototype combined HAT/malaria RDT for HAT screening and malaria detection was evaluated in comparison with an individual RDT for *P. falciparum* malaria detection (the SD BIOLINE ® Malaria Ag *P.f.* RDT) and an individual RDT for gHAT detection (the SD BIOLINE ® HAT 2.0 RDT). The blood samples used in this evaluation were collected in endemic and non-endemic areas of DR Congo (Kwilu province, endemic for HAT and North Kivu province, non-endemic for HAT) and Uganda (Arua province, endemic for HAT and Wakiso province, non-endemic for HAT). Due to the low prevalence of gHAT in the study population in HAT endemic areas, these field-collected blood samples were used to assess the specificity of the prototype RDT for gHAT detection and additional samples collected previously in DRC, Angola and CAR for other studies conducted in the past, frozen and preserved, were used to complete the evaluation of the combined HAT/malaria prototype RDT for gHAT in comparison to the SD BIOLINE ® HAT 2. 0 RDT. The performance of the different tests was evaluated in terms of sensitivity, specificity, accuracy and predictive value for the detection of each of the 2 diseases (HAT and malaria).

Using various innovative diagnostic tools, including SD BIOLINE® HAT RDT, malaria RDT, fluorescence microscopy and LAMP, we developed a diagnostic strategy to intensify HAT screening in the province of Kongo Central, an isolated and circumscribed HAT focus with a relatively small but steady number of cases, to prefigure the elimination of HAT. The strategy was developed on the basis of enhanced passive case detection, complemented by reactive HAT screening. All health facilities in the endemic districts of Central Kongo Province were equipped with RDTs for malaria and HAT to screen for HAT in all suspects who were RDT negative for malaria or unsuccessfully treated for malaria. At least one health facility per district was upgraded to perform microscopy and a reduced number of facilities was upgraded to perform the LAMP molecular test on all unconfirmed RDT-positive suspects for HAT. This passive detection strategy was complemented by reactive screening in villages where HAT was detected by this passive screening strategy.

The performance of the strategy for the elimination of HAT in the Kongo Central HAT focus was evaluated/measured by (i) the number of cases detected and the proportion of gHAT cases detected at the early stage through passive screening before the implementation of the strategy set up compared to the results obtained during the implementation of the strategy under evaluation; (ii) the coverage as the proportion of the population within 5-8 km of a health facility screening for HAT by RDT, (iii) the use of RDT for HAT at different levels, (iv) the proportions of serological suspects from RDT sites that were successfully referred for confirmation to microscopy sites for diagnostic confirmation.

According to above described, the overall methodology was developed with the aim to contribute to HAT diagnostic improvement towards HAT elimination.

1. Human African trypanosomiasis in the Democratic Republic of the Congo: disease distribution and risk



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Abstract

Background:

For the past three decades, the Democratic Republic of the Congo (DRC) has been the country reporting the highest number of cases of human African trypanosomiasis (HAT). In 2012, DRC continued to bear the heaviest burden of *gambiense* HAT, accounting for 84 % of all cases reported at the continental level (i.e., 5,968/7,106). This paper reviews the status of sleeping sickness in DRC between 2000 and 2012, with a focus on spatio-temporal patterns. Epidemiological trends at the national and provincial level are presented.

Results:

The number of HAT cases reported yearly from DRC decreased by 65 % from 2000 to 2012, i.e., from 16,951 to 5,968. At the provincial level a more complex picture emerges. Whilst HAT control in the Equateur province has had a spectacular impact on the number of cases (97 % reduction), the disease has proved more difficult to tackle in other provinces, most notably in Bandundu and Kasai, where, despite substantial progress, HAT remains entrenched. HAT prevalence presents its highest values in the northern part of the Province Orientale, where a number of constraints hinder surveillance and control.

Significant coordinated efforts by the National Sleeping Sickness Control Programme and the World Health Organization in data collection, reporting, management and mapping, culminating in the Atlas of HAT, have enabled HAT distribution and risk in DRC to be known with more accuracy than ever before. Over 18,000 locations of epidemiological interest have been geo-referenced (average accuracy ≈ 1.7 km), corresponding to 93.6 % of reported cases (period 2000–2012). The population at risk of contracting sleeping sickness has been calculated for two five-year periods (2003–2007 and 2008–2012), resulting in estimates of 33 and 37 million people respectively.

Conclusions:

The progressive decrease in HAT cases reported since 2000 in DRC is likely to reflect a real decline in disease incidence. If this result is to be sustained, and if further progress is to be made towards the goal of HAT elimination, the ongoing integration of HAT control and surveillance into the health system is to be closely monitored and evaluated, and active case-finding activities are to be maintained, especially in those areas where the risk of infection remains high and where resurgence could occur.

Keywords: Human African trypanosomiasis, HAT, Sleeping sickness, *Trypanosoma brucei gambiense*, Democratic Republic of the Congo, DRC

Background

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a tropical disease caused by protozoa of the Genus *Trypanosoma*, which are transmitted by the haematophagous tsetse flies (Genus: *Glossina*). Two forms of sleeping sickness are distinguished: one is found in western and central Africa, its causative agent is *T. brucei gambiense* and its progression is generally characterized by a long paucisymptomatic phase that can last several years; the other, caused by *T. b. rhodesiense*, is endemic to eastern and southern Africa and it is characterized by a much more rapid onset of overt symptoms, as well as a faster progression. Both forms almost invariably lead to death, unless appropriate treatment is provided.

In the Democratic Republic of the Congo (DRC), HAT is caused by *T. b. gambiense*, for which humans are the main reservoir. *T. b. rhodesiense*, which is characterized by a significant animal reservoir, is not reported from DRC, but it is present in neighbouring Tanzania, along the eastern shores of Lake Tanganyika [1].

The control of the *gambiense* form of HAT hinges mainly on mass screening of at-risk populations, passive detection and treatment of infected individuals. Targeted vector control can contribute to disease control, especially in areas of intense transmission, by reducing vector density and hence vector-human contact [2].

Despite the high level of control achieved in the sixties [3], for the past three decades DRC has been the country reporting the highest number of sleeping sickness cases [2, 4]. In particular, following the sudden termination of the Belgian bilateral aid in 1990, and in a context of insecurity and general breakdown of the Congolese health system, the disease flared up [5], reaching alarming levels in the second half of the 1990s. At that point in time, over 25,000 new cases were being reported annually. The tide was only reversed when Belgian bilateral aid was resumed in 1998, and large scale screening activities and treatment programmes were restarted [6].

To date, DRC continues to bear the heaviest burden of *gambiense* HAT, having reported 84 % of all African cases in 2012 (i.e., 5,968/7,106). Therefore, achieving the international goal of *gambiense* HAT elimination [7] will depend to a large extent on the progress that DRC will be able to make.

In this paper we review the status of sleeping sickness in DRC from 2000 to 2012, with a focus on spatio-temporal patterns. Epidemiological trends at the national and provincial level are provided. National and provincial maps of HAT distribution are presented, thus adding to previously published information on disease distribution in other HAT endemic countries [1, 8]. Risk maps at the national and provincial levels are also presented for two five-year periods (2003–2007 and 2008–2012). Lastly, a range of control data are presented, which enable disease distribution and risk to be better understood and interpreted.

The study was conducted in the framework of the Atlas of HAT, an initiative of the World Health Organization (WHO), jointly implemented with the Food and Agriculture Organization of the United Nations (FAO), in the framework of the Programme Against African Trypanosomosis (PAAT) [1].

Material and methods

Sources

The main source of data for this study was the National Sleeping Sickness Control Programme—NSSCP (*Programme National de Lutte contre la Trypanosomiase Humaine Africaine*), which provided information on active screening activities conducted by mobile teams, as well as data on passive detection carried out by the Centres for Diagnosis, Treatment and Control (*Centre de diagnostic, traitement et contrôle*—CDTC) operated by the NSSCP and by health referral centres. The NSSCP structure in DRC presently includes a national coordination unit based in Kinshasa and eleven provincial/sub-provincial coordination units. Due to their size, the provinces of Bandundu and Equateur split provincial coordination into two sub-provincial units (i.e., north and south). By contrast, the provinces of Katanga and Maniema are managed by the same coordination unit. Sankuru, a district of difficult access in the Kasai-Oriental province, is managed since 2011 by a separate coordination unit based in the town of Wembo Nyama.

Between 2000 and 2012, each provincial or sub-provincial coordination unit operated between 1 and 13 specialized mobile teams, for an average total of 39 mobile teams/year for the whole country. At the end of 2012, 34 mobile teams were active in the following provinces/ sub-provinces (number of mobile teams in brackets): Bandundu Nord (7), Bandundu Sud (6), Bas-Congo (1), Equateur Nord (5), Equateur Sud (1), Kasai-Occidental (3), Kasai-Oriental (4), Katanga-Maniema (1–1), Kinshasa (1), Province Orientale (3, out of which 2 from *Médecins Sans Frontières* (MSF) acting in the North (Dingila and Ango) and 1 from PNLTHA based in Isangi, in the South) and Sankuru (1).

Additional data were provided by the Non-Governmental Organization (NGO) MSF. MSF's areas of intervention during the study period were located in the Equateur province and, most notably, in the Province Orientale, where for long periods insecurity has compounded remoteness in constraining the action of the NSSCP [9].

Two types of input data were used: (1) annual reports compiled by the national coordination unit, and containing province-level summaries for a range of HAT control and surveillance data, and (2) village-level data generated by the provincial and sub-provincial coordination units and NGOs. The former input files were used to investigate epidemiological trends at the national and provincial levels from 2000 to 2012, while the latter were the basis for building a geospatial database of HAT in DRC, and subsequent HAT mapping and risk assessment.

Mapping the geographic distribution of HAT

The distribution of HAT cases and HAT active screening activities in DRC was mapped using methods developed for the Atlas of HAT [1, 10]. The presented distribution data cover the period 2000–2012, and they are based on the latest update of the Atlas of HAT (reference date: 6 March 2015).

The input data used to build the HAT geospatial database were typically represented by digital spreadsheets. The spreadsheets normally reported the monthly number of new HAT cases and of people screened, distinguishing between active and passive detection. The village name and the related administrative units were also present in the input data, including the *Zone de Santé* (Health Zone) and the *Aire de Santé* (Health Area), which constitute the two territorial subdivisions of the Congolese health system. The *Zone de Santé* covers between 100 and 150 thousand people, whereas the *Aire de Santé* normally covers an average of 10 thousand people in urban areas and 5 thousand people in rural areas. Information on the stage of the disease (i.e., the first—haemolymphatic, versus the second—meningoencephalitic) was only specified in the village-level input files provided by MSF (i.e., 4 % of HAT cases). By contrast, stage information was fully provided at province-level in the NSSCP annual reports. In the case of active screening activities, the number of people living in the screened village (i.e., census) was normally reported in the village-level input datasets (approximately 72 % of screening events).

In geo-referencing villages, the gold standard was the Global Positioning System (GPS), which is frequently used by the NSSCP. In addition to GPS, coordinates were also derived from a variety of sources, most notably from the GEOnet Names Server and other online gazetteers [1]. Qualitative information generated by NSSCP field staff also provided substantial input for geo-referencing.

To enable analysis in a Geographic Information System (GIS), input files were harmonized and imported in a geographic database, including all fields required for the continental Atlas of HAT (i.e., year, census, number of people screened, number of new HAT cases, surveillance type—either active or passive—parasite subspecies, disease stage—either first or second—country name, location name, location coordinates, mapping accuracy and sources).

In addition to the above standard fields for the Atlas of HAT, a few information items specific to DRC were also imported in the database, with a view to facilitating its utilization at the national level.

For the geographical locations (e.g., villages), additional information included the names of all reported administrative units (i.e., province, district, territory, sector/*collectivité*, grouping, village and neighbourhood), as well as the names of the health system units (i.e., *Zone de Santé* and *Aire de Santé*). For data related to active screening activities, the name of the mobile team was also retained.

Although most HAT cases are reported from rural areas, a sizeable number of urban residents are also affected. This phenomenon is generally related to city dwellers who frequently visit neighbouring rural areas, as studied in some detail in the city of Kinshasa [11–15].

For all mapped HAT cases and geographic locations, mapping accuracy was estimated with methods already described [1].

Risk mapping

The broad patterns of HAT risk at the continental level have already been presented elsewhere [8, 16]. The present paper is focused on the risk in DRC over two five-year study periods (2003–2007 and 2008–2012), and it also includes Province-level risk maps. Periods of 5 years have been identified as particularly interesting to monitor the elimination of *gambiense* HAT [2, 8].

The methodology used to estimate and map the risk of HAT has already been described [16, 17]. In essence, a risk function is estimated through kernel smoothing [18], which enables GIS point layers to be converted into continuous intensity surfaces. In this context, intensity is defined as the number of events per unit area [19]. Two smoothed surfaces are derived, one for HAT cases and one for human population. Landscan databases constitute the source of human population data [20, 21]. The ratio of the two intensity surfaces defines the risk function 'R'. Thresholds are applied to the risk function in order to distinguish different categories of risk, ranging from 'very high' to 'very low' (Table 1). Finally, Landscan population database is used to calculate the number of people at risk at the end of the study periods (2007 and 2012 respectively).

In addition to geo-referenced HAT cases, also the cases not yet mapped at the village-level were used in the risk analysis. The latter include 5,397 cases (i.e., 7 % of the total reported in 2003–2012), and they were distributed among mapped locations by means of proportional allocation [17]. In particular, 2,579 cases (i.e., 48 % of the total unmapped) were allocated by using information on the '*Aire de santé*', whose radius rarely exceeds 6 km. For 493 cases (9 % of the total unmapped), for which information on the corresponding '*Aire de santé*' was unavailable, the '*Zone de Santé*' was used instead (average size 4,600 km^2). The remaining 2,325 cases (i.e., 43 % of the total unmapped) were allocated at coarser administrative levels.

2.1 Table 1. Thresholds for the definition of sleeping sickness risk categories.

Thresholds are applied to the risk function R (i.e., the ratio between the average annual intensity of HAT cases and the intensity of exposed population). Where the risk function is $< 10^6$ (i.e., < 1 HAT case per 10^6 people per annum), risk is considered 'marginal' [17]

Category of risk	R	HAT cases per annum
Very high	$>10^2$	1 per 10^2 people
High	$10^3 < R < 10^2$	1 per 10^3 people AND < 1 per 10^2 people
Moderate	$10^4 < R < 10^3$	1 per 10^4 people AND < 1 per 10^3 people
Low	$10^5 < R < 10^4$	1 per 10^5 people AND < 1 per 10^4 people
Very low	$10^6 < R < 10^5$	1 per 10^6 people AND < 1 per 10^5 people

Results

Epidemiological trends, 2000–2012

National level

The number of new HAT cases reported yearly from DRC decreased by 65 % from 2000 to 2012, i.e., from 16,951 to 5,968. The slight increase observed between 2011 and 2012 is to be ascribed to intensive active case detection in the Bandundu province aimed at the enrolment of HAT patients in clinical trials.

In the same period a similar decrease was observed in cases detected by both passive (73.3 %) and active screening (56.7 %), as shown in Table 2. Interestingly, infection rate (i.e., cases detected/people screened) in active screening decreased by 58 % during the first four years of the study period, but it remained fairly stable thereafter. Infection rate in passive screening decreased by 82.3 % between 2000 and 2008, while stagnation has been observed during the last six years of the study period.

As concerns the intensity of surveillance, from 2005 a decrease was observed in the number of people actively screened, only partly offset by a sustained increase in people passively screened.

In active screening activities the disease stage ratio (i.e., the ratio between the number of cases detected in second stage and those detected in first stage) has decreased by 37.3 % (from 0.67 in 2000 to 0.42 in 2012). As expected, higher stage ratios characterized passive detection throughout the whole study period. However, in relative terms, a similar decrease was observed (46.9 %), with a minimum of 1.33 in 2011.

The ratio between passively to actively detected cases has hovered around one throughout the study period, except for 2001, 2009 and 2012 when it was 0.68 %, 0.67 % and 0.59 % respectively (Table 2). Average attendance rates in active screening activities remained fairly high and stable throughout the study period, with an average of 78.6 % (Table 3).

Provincial level

As shown in Fig. 1, national averages presented in Table 2 hide substantial variation at the provincial level. Table 4 shows the number of new HAT cases reported between 2000 and 2012 by the different Provinces, while Additional file 1 presents attendance rates during active screening activities—which ranged from 73.1 % in Kasai to 86.6 % in Province Orientale. More detailed data on HAT control and surveillance in the different provinces of DRC are provided in Additional file 2. From these data, the main provincial trends can be deduced.

Bandundu Province, which covers an area of 296,500 km^2 (12.6 % of DRC), accounts for the largest share of cases reported since 2001 (i.e., ≈ 47.6 %). The Province was divided in 2004 into two sub-provincial coordination units, i.e., Bandundu Nord, located in the city of Bandundu, and Bandundu Sud, located in the city of Kikwit. Bandundu has been the recipient of almost half of the budget for HAT control in DRC during the last ten years (NSSCP, unpublished). This commitment enabled to maintain a substantial and sustained effort in terms of active case finding, including what the NSSCP calls "*action d'envergure*", in which several mobile teams focus on a highly endemic "*zone de santé*". On average, more than 800,000 people were screened in Bandundu every year. On the other hand, in 2009 the province initiated the process of integrating HAT control and surveillance in the health system.

Data on passive screening in Bandundu differ from the national trends. Whereas the decrease in cases detected by passive screening experienced at national level has been of 73.3 %, in Bandundu it has been of 53.7 %. If measured in terms of the number of cases detected, the epidemiological situation in Bandundu improved over the study period, with a reduction of 43 %; however, this reduction was below the national average (65 %).

Bas-Congo is relatively small a province (i.e., 54,400 km^2), which also benefits from a good transport network. This facilitates HAT control activities. In addition, proximity to NSSCP headquarters in Kinshasa makes logistical support to the provincial coordination unit easier. The decline in number of cases, which at 87 % was sharper than the 65 % national average, can largely be ascribed to these comparative advantages. In 2009 the improvements in the epidemiological situation led to initiate the process of integration of HAT into the health system, with the subsequent reduction in the number of mobile teams (from five at the beginning of the study period to one in 2011). The mobile team presently operational plays a role that is largely reactive, visiting areas where passive detection shows an increase in the number of detected cases.

Equateur is a vast province of 395,700 km^2 that was split into two sub-provincial coordination units—Equateur Nord based in Bwamanda and Equateur Sud based in Mbandaka. The disease is mainly prevalent in the northern part of the province.

2.2 Table 2. HAT control and surveillance in DRC (2000–2012)

Year	Active screening							Passive surveillance							Total	
	People screened	HAT Cases	Infection rate	Stage				People screened	HAT Cases	Infection rate	Stage				HAT Cases	Passive cases/ active cases
			[%]	P1	P2	N.a[a]	P2/P1			[%]	P1	P2	N.a[a]	P2/P1		
2000	1,442,951	8,679	0.60	4,958	3,337	384	0.67	162,855	8,272	5.08	1,663	6,449	160	3.88	16,951	0.95
2001	1,936,755	10,286	0.53	6,013	3,379	894	0.56	91,483	7,014	7.67	1,269	5,507	238	4.34	17,300	0.68
2002	1,925,443	7,298	0.38	4,328	2,638	332	0.61	114,911	6,518	5.67	1,308	5,206	4	3.98	13,816	0.89
2003	2,288,071	5,781	0.25	3,359	2,234	188	0.67	117,887	5,678	4.82	980	4,679	19	4.77	11,459	0.98
2004	2,360,962	5,226	0.22	3,173	1,935	118	0.61	163,694	5,113	3.12	844	4,259	10	5.05	10,339	0.98
2005	2,310,722	4,919	0.21	3,189	1,590	140	0.50	151,497	5,330	3.52	1,031	4,246	53	4.12	10,249	1.08
2006	2,227,855	3,749	0.17	2,471	1,141	137	0.46	214,396	4,264	1.99	879	3,373	12	3.84	8,013	1.14
2007	1,984,643	4,142	0.21	2,810	1,218	114	0.43	217,561	4,013	1.84	911	3,074	28	3.37	8,155	0.97
2008	1,452,082	3,692	0.25	2,539	1,102	51	0.43	573,972	3,626	0.63	945	2,666	15	2.82	7,318	0.98
2009	1,876,160	4,290	0.23	3,316	847	127	0.26	183,554	2,888	1.57	706	2,149	33	3.04	7,178	0.67
2010	1,740,534	2,802	0.16	2,016	734	52	0.36	278,606	2,822	1.01	849	1,966	7	2.32	5,624	1.01
2011	1,189,603	2,652	0.22	2,073	538	41	0.26	234,632	2,938	1.25	1,256	1,669	13	1.33	5,590	1.11
2012	1,497,047	3,759	0.25	2,592	1,099	68	0.42	237,319	2,209	0.93	719	1,480	10	2.06	5,968	0.59
Total	24,232,828	67,275	0.28	42,837	21,792	2,646	0.51	2,742,367	60,685	2.21	13,360	46,723	602	3.50	127,960	0.90
Average	1,864,064	5,175	0.28	3,295	1,676	204	0.48	210,951	4,668	3.01	1,028	3,594	46	3.45	9,843	0.93

[a]N.a. = Not available

The southern part is characterized by low human population density as well as by numerous rivers and dense vegetation hindering access. Large areas in Equateur Sud are beyond the reach of NSSCP teams, and some communities are extremely isolated (e.g., pygmies). Nevertheless, in this context, targeted HAT surveys have not shown worrying results. Cases reported from Equateur Sud account for only 7 % of the total cases reported at the provincial level. Subsequently, trends in Equateur province are to a large extent those observed in the northern part.

2.3 Table 3. Attendance rate in HAT active screening activities in DRC (2000–2012)[a]

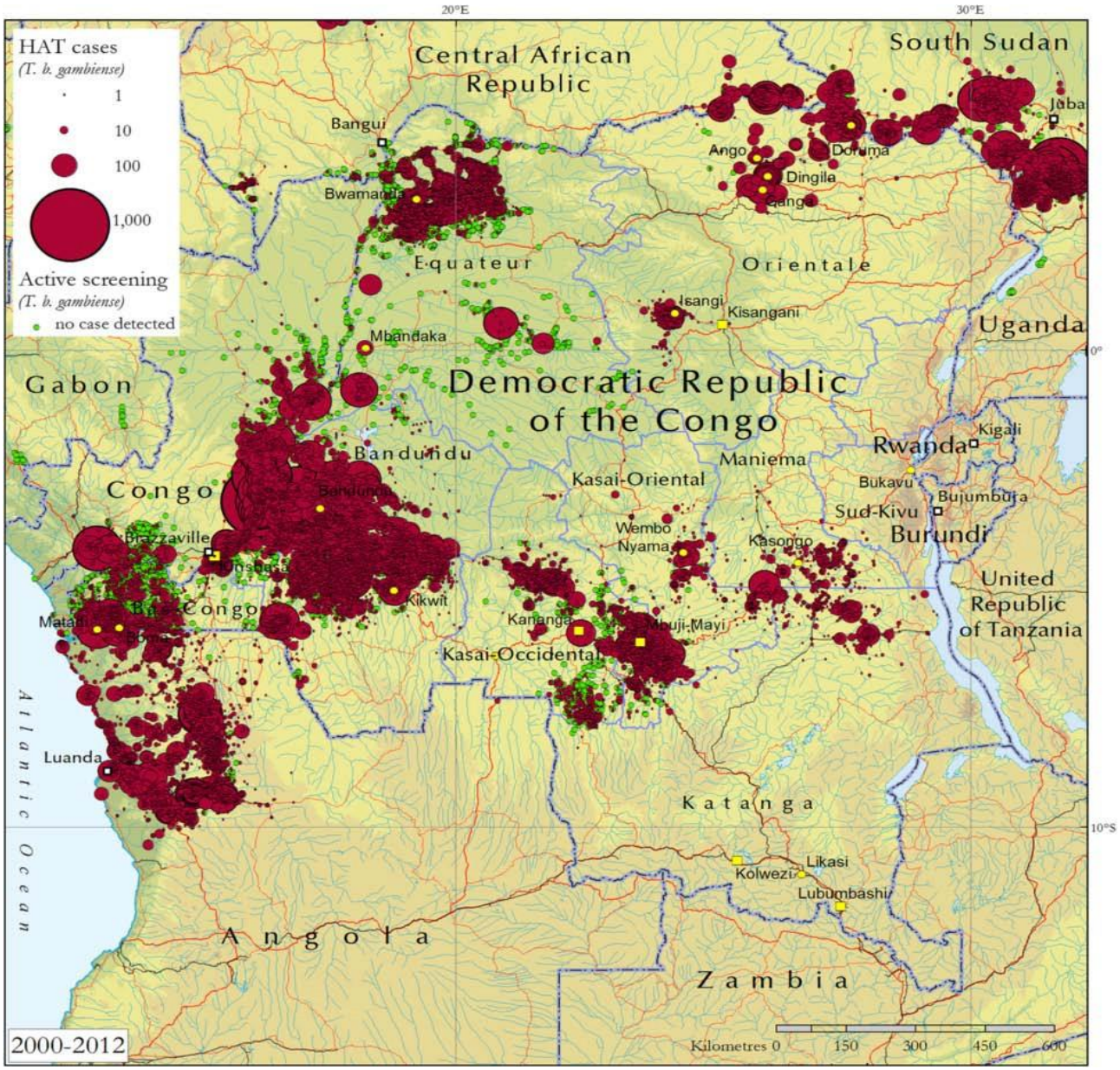
Year	Mobile teams [number]	Census (in actively screened locations)[b]	Attendance rate [%]
2000	33	1,981,804	72.81
2001	33	2,493,959	77.66
2002	43	2,553,761	75.40
2003	46	2,780,623	82.29
2004	47	2,795,687	84.45
2005	45	2,806,751	82.33
2006	45	2,785,726	79.97
2007	38	2,347,330	84.55
2008	34	2,329,327	62.34
2009	33	2,413,026	77.75
2010	35	2,350,939	74.04
2011	34	1,367,989	86.96
2012	34	1,820,520	82.23
Average	38	2,371,342	78.67

[a]People actively screened is shown in Table 2

[b]Collected by mobile teams

In Equateur Nord, a spectacular reduction in the number of reported cases has been observed over the study period. In 2000, Equateur was the major contributor to the total HAT cases reported in DRC (i.e., 40.2 %), whereas in 2012 its share plummeted to 3.3 %, corresponding to a reduction of 97 % in reported cases. These results were achieved in spite of remoteness, and they were made possible by the important support of financial partners, as well as the commitment of staff, the judicious management and the effective partnership between the NSSCP and the NGO (Memisa) in charge of implementation. The Equateur province has initiated the process of integration of HAT control and surveillance into the health system. In recent years, despite the majority of CDTC having been closed, most reported cases were detected by passive screening and a subsequent increase in the proportion of second versus first stage cases was observed. Very close monitoring of the performance of the health system is imperative in this province, especially because attendance rate in

active screenings has fallen below 50 %. The rate of utilization of fixed health facilities also needs to be monitored.



2.1 Figure 1. HAT reported cases in DRC (2000–2012)

Kasai-Oriental and Kasai-Occidental were managed by a single provincial coordination unit until 2005, when coordination was split between Mbuji-Mayi (for Kasai-Oriental) and Kananga (for Kasai-Occidental). Access to HAT foci is generally difficult because of the poor conditions of the road network. Access is especially problematic in Kasai-Occidental because of the distance between Kananga and the HAT foci. By contrast, the

foci in Kasai-Oriental are located around Mbuji-Mayi, which simplifies the activities of the mobile teams. In addition, the epidemiological significance of these two provinces — which, when combined, represent the second contributor to HAT cases in DRC after Bandundu — brought about a number of operational research projects. These contributed to upgrading logistics, heath care facilities and human resources, and thus boosted control activities. At the same time, intense traditional mining of diamonds continues to facilitate disease transmission, whilst also limiting participation in active screening surveys.

2.4 Table 4. *T. b. gambiense* Sleeping Sickness in the Provinces of DRC: new cases reported between 2000 and 2012

Province		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
Bandundu (Nord and Sud)		5,586	7,179	6,261	5,367	4,604	4,812	3,986	3,826	3,916	4,456	2,923	2,502	3,167	58,585
	Bandundu Nord	3,138	3,989	3,630	2,568	2,540	2,746	1,926	1,677	2,044	2,061	1,409	1,433	2,047	31,208
	Bandundu Sud	2,448	3,190	2,631	2,799	2,064	2,066	2,060	2,149	1,872	2,395	1,514	1,069	1,120	27,377
Bas-Congo		792	719	739	517	266	201	172	110	196	195	164	104	106	4,281
Equateur (Nord and Sud)		6,812	5,096	2,594	1,700	1,320	902	691	657	552	425	308	321	197	21,575
	Equateur Nord	6,673	4,990	2,436	1,597	1,250	824	572	532	418	270	184	219	107	20,072
	Equateur Sud	139	106	158	103	70	78	119	125	134	155	124	102	90	1,503
Kasaï (Occidental and Oriental)		2,811	2,905	3,173	2,606	2,720	2,766	2,115	1,896	1,431	1,376	967	1,054	872	26,692
	Kasaï-Occidental	-	-	-	-	-	618	515	575	357	386	270	356	171	-
	Kasaï-Oriental	-	-	-	-	-	2,148	1,600	1,321	1,074	990	697	698	701	-
Katanga and Maniema		323	709	589	728	667	391	355	368	218	137	122	115	218	4,940
	Katanga	-	-	-	-	-	108	80	99	0	66	52	62	91	-
	Maniema	323	-	-	-	-	283	275	269	218	71	70	53	127	-
Kinshasa		627	685	459	286	331	256	228	399	238	198	153	143	134	4,137
Province Orientale		0	5	0	251	431	920	463	897	767	391	986	1,351	1,273	7,735
Sud-Kivu		0	2	1	4	0	1	3	2	0	0	1	0	1	15
Total		16,951	17,300	13,816	11,459	10,339	10,249	8,013	8,155	7,318	7,178	5,624	5,590	5,968	127,960

Recently, a decrease in the trade of diamonds has caused some people to quit mining in riverine, forested environments, and to return to their villages of origin for farming. This may pose an increased risk of transmission in some silent areas, and monitoring of the performance of the active and passive detection system is therefore needed.

Access from Mbuji-Mayi is only difficult to the northern part of Kasai-Oriental (i.e., Sankuru), which in the past was managed by the provincial coordination in Maniema. In 2011, this region has been established as a new sub-provincial coordination unit, with its base in the city of Wembo Nyama. For the purpose of this study, data from Sankuru were still included in those from Kasai Oriental.

Kasai is the only province where the number of people screened by mobile teams more than doubled over the study period, while the trend in reported cases is in line with the national average (i.e., 69 % reduction).

Katanga and Maniema share a border as well as transboundary HAT foci. This proximity and the relatively low number of cases reported enable a single provincial coordination unit based in Kasongo (Maniema) to manage both Provinces. All foci are surveyed by two mobile teams, one for each Province. Logistics is difficult because of the large distance from NSSCP headquarters in Kinshasa and for the very poor conditions of the roads. Insecurity also constrains access to transmission areas, which contributes to confounding the epidemiological picture in these provinces. Knowledge gaps notwithstanding, the generally low endemicity, combined with a 32 % reduction in HAT reported cases, led to initiate the process of integration of HAT control and surveillance into the health system. This allowed to shift mobile teams to Sankuru. In this context, close monitoring and evaluation of the health system performance in detecting HAT cases is warranted. As already observed for Equateur province, most of the cases from Katanga and Maniema are detected by passive detection, with an increase in the disease stage ratio in recent years.

During the study period the provincial coordination unit in Kasongo (Maniema) has reported a few (six) cases originating from Sud Kivu. Nevertheless, there is reason to believe that these individuals, although administratively associated to Sud Kivu, are likely to have been infected in the transmission areas in Maniema, where they conduct their main activities (V. Kande personal communication).

Kinshasa Province includes the urban and the rural Kinshasa for a total area of 10,500 km2. A provincial coordination is based in the city of Kinshasa. The rural Kinshasa covers mainly the north-eastern area of the province (i.e., the communes of Maluku and Nsele). Although under the administrative umbrella of Kinshasa, farming is the main activity and the setting is the same we can observe in other rural areas of DRC. As concerns urban Kinshasa, favourable ecosystems where transmission can occur do exist (e.g., along the many rivers that cross the city). Moreover, many residents of Kinshasa have strong commercial and recreational connections with HAT transmission areas, especially in neighbouring Bandundu province.

In Kinshasa province as a whole, improvements in the epidemiological situation were observed during the study period, with a 79 % reduction in the number of cases. However, case detection largely relies on passive detection, and most cases continue to be detected in stage 2.

The Province Orientale is a vast area of 502,900 km2. It is affected by serious security issues, mainly in its northern part, weak transportation infrastructure, and remoteness from NSSCP headquarters in Kinshasa. The provincial coordination unit is based in Isangi, and its location only enables it to cover transmission areas in the southern part of the province. Epidemiological trends are particularly difficult to interpret because of the above mentioned constraints to HAT control and surveillance activities [9]. Orientale is therefore the province where knowledge of the epidemiological situation is the least complete. Only the more accessible, southern

part of the province has benefited from sustained HAT control since 2004. Data for the period 2000–2002 are particularly scanty, whilst infection rates found in following years indicate that transmission was very intense in some areas. Despite gaps in the epidemiological record, it is clear that this region has shown the highest HAT prevalence in the last years in the whole of DRC, especially in a few Zones de Santé located in the northern part of the province (e.g., Ganga-Dingila, Ango and Doruma). Between 2007 and 2014, MSF established a project implementing a control programme. Despite initial stumbling blocks caused by insecurity, the project managed to sustain its activities and the knowledge of HAT epidemiology in the province—as well as disease control—improved.

The geographic distribution of HAT, 2000–2012

As we write, 119,634 HAT cases (93.6 % of the total reported in the period 2000–2012) and 18,072 locations (86.8 % of the total reported) have been mapped in DRC (Fig. 1) (Atlas of HAT reference date: 6 March 2015). The estimated average mapping accuracy is 900 m and 1,700 m for reported HAT cases and reported geographic locations respectively. The results of mapping at the regional level and the details on the geographical accuracy of mapped villages are summarized in Additional file 3. Provincial maps of HAT distribution for the period 2000–2012 are provided in Additional file 4. Furthermore, Additional file 5 provides separate maps for the periods 2003–2007 and 2008–2012 (both at the national and provincial level).

The risk of HAT, 2003–2007 and 2008–2012

The results of the risk analysis are mapped in Fig. 2 and summarized in Figs. 3 and 4. Additional file 6 provides province level details for areas and populations at risk, and the related province level maps are available from Additional file 7.

Taking the latest study period as a reference (i.e., 2008–2012), less than one third of the land area in DRC (i.e., 715 thousand km2) and approximately half of the population (i.e., 36.6 million) are estimated to be at various levels of HAT risk.

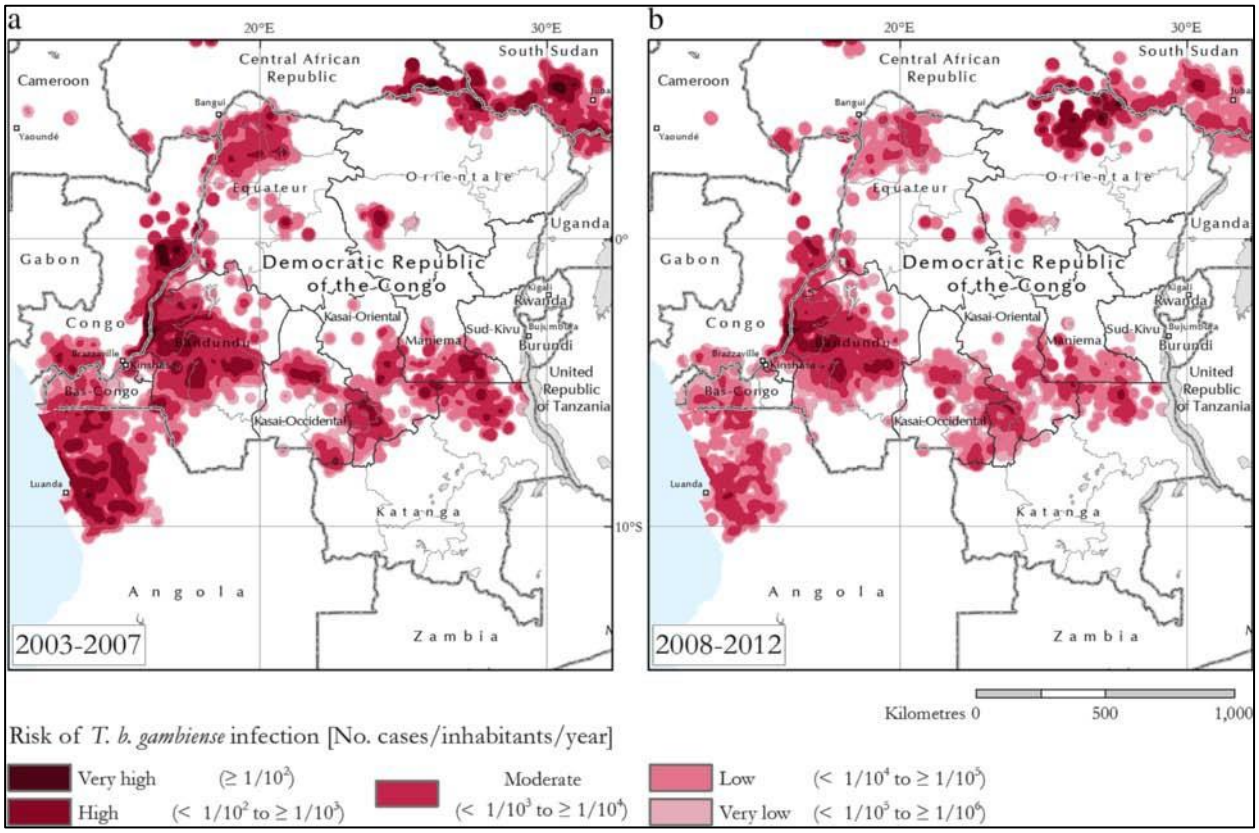
Areas at ‘very high’ to ‘high’ risk account for a relatively small proportion, i.e., ≈ 8 % of the total at-risk area (down from 12 % in 2003–2007), and ≈ 4 % of the total at-risk population (down from 8 % in 2003–2007). As shown in the country-level risk map in Fig. 2b and in the province-level risk maps (Additional file 7), these high risk areas are found mainly in Bandundu (Zones de Santé Kwamouth, Bolobo, Mushie, Bandundu, Nioki, Bokoro, Bagata, Kikongo, Kenge, Yasa Bonga, Masi Manimba, Mosango, Bulungu, Kimptutu, Ipamu, and Lusanga), Equateur (Zones de Santé Bikoro, Ntondo and Iboko), and Province Orientale (Zones de Santé Ganga-Dingila, Doruma, Ango, Bili and Poko). With the exception of Bas-Congo, Maniema and Sud and Nord Kivu, high risk areas are also present in all other provinces, including Kasai-Oriental (Zones de Santé Bibanga, Lubao, Tshitshimbi, and Kabinda) and Kasai-Occidental (Zones de Santé Kakenge), Katanga (Zones de Santé Nyunzu and Mbulula) and Kinshasa (Zones de Santé Maluku I).

Risk is estimated to be 'moderate' in sizable areas of Bandundu, Equateur and a number of other Provinces, accounting for ≈ 31 % of the total at-risk area (down from 38 % in 2003–2007), and ≈ 23 % of the at-risk population (down from 32 % in 2003–2007).

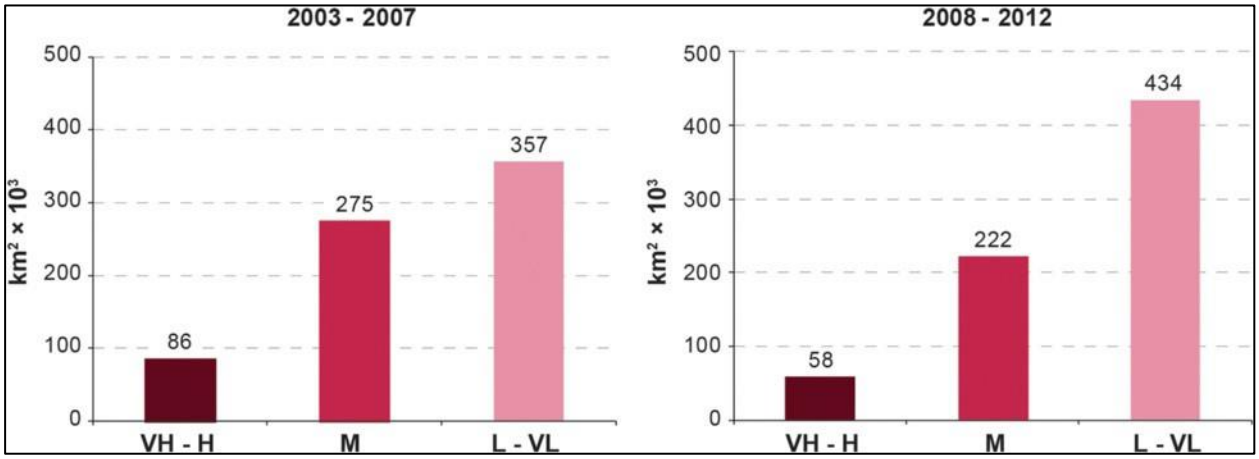
Although the total population at risk has increased by 10 % between the two study periods, mainly due to population growth, the intensity of HAT risk has decreased. In particular, the population at high and very high risk has decreased by 45 %—from 2.8 to 1.5 million. The population at moderate risk also decreased from 10.5 to 8.2 million (i.e., 21 % reduction). This national trend is not followed by Province Orientale, where the improvement in security conditions during the period 2008–2012 allowed access to pockets of previously unsurveyed populations. This resulted in an increase of 86 % in the population at very high, high and moderate risk.

Discussion

Despite the fluctuations in intensity and variations in effectiveness of HAT control and surveillance activities, the number of new HAT cases reported from DRC to WHO has decreased almost every year in the period 2000–2012, with a total reduction of 65% (from 16,951 to 5,968). Although the downward national trend of reported cases is considered extremely positive, it is challenging to discuss national-level data for such a vast country as DRC. In depth analyses at the provincial and sub provincial levels, beyond the scope of the present paper, would be needed.

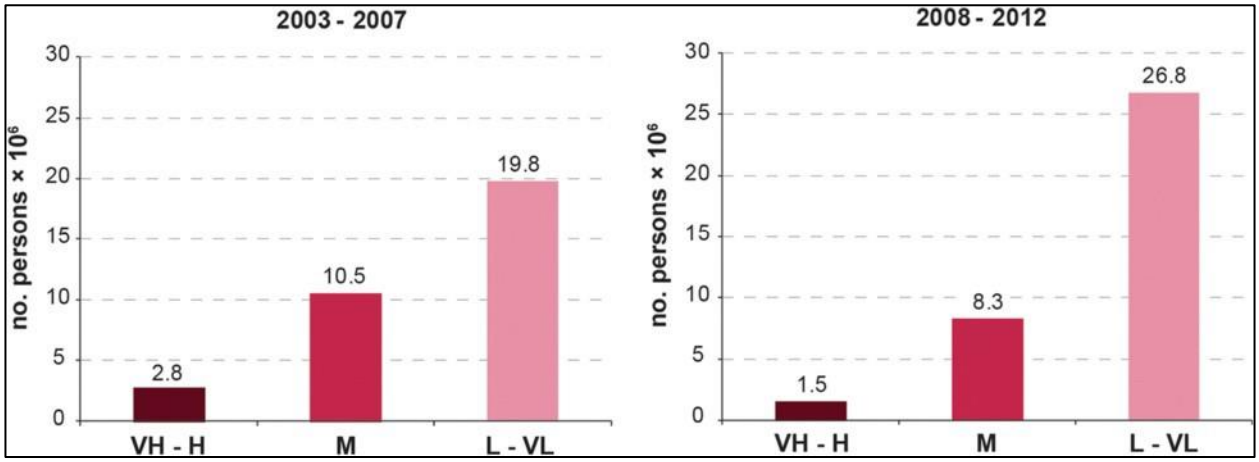


2.2 Figure 2. The risk of T. b. gambiense infection in DRC, a 2003–2007 and b 2008–2012



2.3 Figure 3. The areas at risk of T. b. gambiense infection in DRC (km2 × 103). Periods 2003–2007 and 2008–2012.
VH: Very High; H: High; M: Moderate; L: Low and VL: Very Low

The average attendance rate during active case-finding surveys, based on census data collected by mobile teams, has been stable around 79 %. Although mobile teams can not cover all areas of disease transmission, this figure indicates that they are at least in a position to detect most of the HAT infections occurring in their area of action. This attendance rate is in line with estimates for the period 1997–1998 [22].



2.4 Figure 4. The population at risk of *T. b. gambiense* infection (no. persons × 106). Periods 2003–2007 and 2008–2012.
VH: Very High; H: High; M: Moderate; L: Low and VL: Very Low

On the other hand, the ratio between passively versus actively detected cases has remained stable at around one. This can be related to one negative aspect (i.e., inability of active case-finding surveys to cover all transmission areas), but also to a positive one (i.e., capacity of passive surveillance to detect cases that escape active screening). Also, looking at the generally decreasing trend in the stage ratio (i.e., P2/P1), it appears that the capacity to detect cases in the early stage has improved. Concerning cases passively detected, it was unfortunately not possible to differentiate between cases detected by specialized NSSCP units like CDTC and those detected by recently involved health care facilities. However, detected passive cases indicate that disease

transmission is still ongoing in certain areas, which deserves an accurate identification and a quick and targeted reaction by mobile teams.

Despite the encouraging national trends summarized above, other observations give reason for concern. First, the intensity of active case-finding surveys is decreasing. Second, the NSSCP had a specialized network of centres for diagnosis and treatment (CDTC), staffed with motivated and skilled professionals; however, the progressive shift of responsibility for passive screening to the health system, while expanding HAT surveillance to more health care facilities, could diminish performance if staff are less motivated and skilled. Last, because of difficult geographical accessibility, security constraints and lack of funds, there still exist transmission areas not covered by adequate control and surveillance activities.

As a result of decreasing disease trends, the integration of HAT control and surveillance into the Health System has been for many years the objective of the NSSCP, and it has more recently found backing in the 'National strategy for the reinforcement of the health system'. The process of integration has been gradually rolled out in most endemic provinces, (except in the insecurity-ridden Province Orientale), either to replace mobile teams in areas where, due to low prevalence, they were neither effective nor sustainable, or to complement active screening in areas where the disease transmission is still high. Since 2009, the reduction of mobile teams and the integration of HAT control and surveillance into selected health care facilities have been mainly implemented in the provinces of Bandundu, Bas-Congo, Maniema, Katanga and Equateur Nord. These provinces have maintained one operational mobile team—except Bandundu (13 mobile teams) and Equateur Nord (five mobile teams)—to act and react in areas of active transmission. The members of the dismantled mobile teams were, for the most part, appointed to health care facilities in charge of passive screening, thus potentially improving the diagnostic performance of these facilities. In the other provinces, the progressive integration of HAT control and surveillance into the health system has advanced while keeping mobile teams operational.

The sustainability and long-term performance of this strategy are faced by numerous threats. It is difficult to replace committed and trained staff in HAT diagnosis and treatment who retire. The loss of the economic incentives associated with active case-finding surveys reduces motivation.

Rapid and effective treatment of newly detected HAT patients is one of the cornerstones of HAT control. Although the topic has not been discussed in the present study, closing down mobile teams and CDTCs may have a dramatic impact on treatment compliance.

A recent survey revealed that 524 fixed health facilities in DRC provide some type of diagnosis for HAT, and that over 30 million people at risk (i.e., 83 % of the total population at risk in DRC) are estimated to live within 5-h travel of such facilities [23]. However, low attendance or access to Health Services [24], coupled with lack of screening tools or skilled staff, would suggest that the real coverage may be much lower than the estimated

potential coverage. As a result, improved performance of passive detection may not be offsetting the decreased intensity of active screening. If this were the case, recent trends in the number of HAT cases could be biased by an overall reduction in detection capability.

In addition, in 2011 and 2012 the mobile teams still operational have seen their activity reduced by 50 % because of financial constraints, thus resulting in the lowest number of people screened by active case-finding surveys in the study period. Cuts have spared a few mobile teams operating in areas of high transmission, and those involved in research projects.

Specific challenges affect HAT surveillance and control in densely populated areas such as urban Kinshasa, where traditional control strategies may not be adequate and innovative, and adaptive approaches might be needed.

Over the last decade DRC has hosted several clinical trials for the development of new screening, diagnostics and treatment tools. Clinical trials had a positive impact on the NSSCP, which can be measured in terms of new tools, as well as reinforced screening activities and increased capacity and expertise among the staff. However, as clinical trials need tailored plans for recruiting patients, they have at times altered the NSSCP's operational plans. Whilst upgrading health care facilities hosting them, clinical trials have often forgotten to support mobile teams looking for patients, and they have failed to foresee the challenges the sites would face after completion of the trials.

Cases of *T. b. rhodesiense* sleeping sickness are reported from Kigoma, in the United Republic of Tanzania, only a few kilometers away from the border with DRC. Despite this, the threat of a merger of transmission areas of the two forms of the disease is not as high as in Uganda. Indeed, whereas cattle movement is the major factor for the northern spread of *T. b. rhodesiense* in Uganda [25], the natural barrier represented by Lake Tanganyika separates the *T. b. rhodesiense* affected areas in Kigoma and the *T. b. gambiense* affected areas in DRC. Although traffic is intense between the two shores of the lake, cattle trade is not involved. In addition, the pattern of *T. b. rhodesiense* transmission in Kigoma is mainly characterized by the wildlife reservoir in a game reserve [26].

Conclusions

The progressive decrease in HAT cases reported in DRC is likely to reflect a real decline in disease incidence. Nevertheless caution is needed when interpreting the reported figures, because a number of weaknesses characterize the control and surveillance of HAT in both passive and active case detection.

The government strategy to reinforce the national health system, together with the decrease in number of cases in several transmission areas, call for the integration of HAT control and surveillance into the existing health care facilities. However, at this juncture it must be carefully avoided to push forward integration when and

where staff are not yet sufficiently skilled or the equipment and monitoring is not assured. There is still a need for both (i) mobile teams, either to tackle areas where intense transmission persists or where passive detection indicates an increase of transmission, and (ii) central structures at provincial and national level, which are needed for technical support (including training), data analysis, planning, monitoring and evaluation of the entire control and surveillance activities at all levels of the health system.

HAT control and surveillance in DRC is still extremely dependent on international aid. Success in controlling HAT in DRC has rested on skilled staff who were committed to providing support to patients, but who were also economically motivated thanks to external funding. Worryingly, during the last years a decrease in external financial support has been observed. Therefore, it seems necessary to question the extent to which the implementation of the present integration strategy is driven by changes in the epidemiological context and by the limited existing capabilities of the national health system, or whether, worryingly, the process is mainly a consequence of financial constraints. Careful assessment and close monitoring and evaluation of the NSSCP strategy in DRC is warranted, in particular in relation to the integration of HAT control and surveillance into a health system widely described as weak. To counter the possible decrease of external funds for HAT control in DRC, it is crucial to increase the ownership of HAT elimination by national health policy makers.

Decentralization could help the management of the NSSCP, but it also poses challenges when it comes to giving HAT sufficient priority in the annual plans of activities at the provincial level, where competition from other health issues is harsh.

To consolidate the recent achievements in disease control and to avoid negative reversals, it seems urgent to explore alternative and adapted control and surveillance strategies. These may benefit from recently developed screening tools, advances in the development of new oral drugs, and an expectedly more favourable security environment.

The lessons learned in DRC are believed to be valuable for a range of countries affected by *gambiense* HAT, and they can therefore contribute to reaching the goals of HAT elimination set at the international level [27].

Additional files

Additional file 1: Attendance rate in HAT active screening activities in the provinces of DRC (period 2000-2012).

Additional file 2: HAT control and surveillance in the Provinces of DRC (2000-2012).

Additional file 3: Progress status of mapping for HAT cases and geographic locations in DRC (period 2000 – 2012), and geographical accuracy of mapped villages.

Additional file 4: The distribution of HAT in the Provinces of DRC (2000-2012).

Additional file 5: The distribution of HAT in the DRC and its Provinces (2003-2007 and 2008-2012).

Abbreviations

CDTC: Centres for Diagnosis Treatment and Control (*Centre de diagnostic traitement et contrôle*); DRC: Democratic Republic of the Congo; FAO: Food and Agriculture Organization of the United Nations; GIS: Geographic Information System; GPS: Global Positioning System; HAT: human African trypanosomiasis; MSF: Médecins Sans Frontières; NGO: Non-Governmental Organization; NSSCP: National Sleeping Sickness Control Programme (PNLTHA: *Programme Nationale de lutte contre la Trypanosomiasis humaine africaine*); PAAT: Programme Against African Trypanosomosis; *T. b*: *Trypanosoma brucei*; WHO: World Health Organization.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CL coordinates the NSSCP in DRC. PPS coordinated the HAT Atlas initiative. GC supervised the technical aspects related to data management and GIS. CL, PPS and GC jointly drafted the manuscript. MP implemented geo-positioning procedures and managed the geo-database underpinning the Atlas of HAT. All authors have contributed to conceptualizing the manuscript, and commented on and approved the final draft.

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Disclaimers

The boundaries and names shown and the designations used on the maps presented in this paper do not imply the expression of any opinion whatsoever on the part of WHO and FAO concerning the legal status of any

country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The views expressed in this paper are those of the authors and do not necessarily reflect the views of WHO and FAO.

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2. Sensitivity and Specificity of a Prototype Rapid Diagnostic Test for the Detection of *Trypanosoma brucei gambiense* Infection: A Multi-centric Prospective Study

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Abstract

Background

A major challenge in the control of human African trypanosomiasis (HAT) is lack of reliable diagnostic tests that are rapid and easy to use in remote areas where the disease occurs. In *Trypanosoma brucei gambiense* HAT, the Card Agglutination Test for Trypanosomiasis (CATT) has been the reference screening test since 1978, usually on whole blood, but also in a 1/8 dilution (CATT 1/8) to enhance specificity. However, the CATT is not available in a single format, requires a cold chain for storage, and uses equipment that requires electricity. A solution to these challenges has been provided by rapid diagnostic tests (RDT), which have recently become available. A prototype immunochromatographic test, the SD BIOLINE HAT, based on two native trypanosomal antigens (VSG LiTat 1.3 and VSG LiTat 1.5) has been developed. We carried out a non-inferiority study comparing this prototype to the CATT 1/8 in field settings.

Methodology/Principal Findings

The prototype SD BIOLINE HAT, the CATT Whole Blood and CATT 1/8 were systematically applied on fresh blood samples obtained from 14,818 subjects, who were prospectively enrolled through active and passive screening in clinical studies in three endemic countries of central Africa: Angola, the Democratic Republic of the Congo and the Central African Republic. One hundred and forty-nine HAT cases were confirmed by parasitology. The sensitivity and specificity of the prototype SD BIOLINE HAT was 89.26% (95% confidence interval (CI) = 83.27–93.28) and 94.58% (95% CI = 94.20–94.94) respectively. The sensitivity and specificity of the CATT on whole blood were 93.96% (95% CI = 88.92–96.79) and 95.91% (95% CI = 95.58–96.22), and of the CATT 1/8 were 89.26% (95% CI = 83.27–93.28) and 98.88% (95% CI = 98.70–99.04) respectively.

Conclusion/Significance

After further optimization, the prototype SD BIOLINE HAT could become an alternative to current screening methods in primary healthcare settings in remote, resource-limited regions where HAT typically occurs.

Author Summary

Early diagnosis and treatment of human African trypanosomiasis is essential for safe and effective treatment. The tests used to screen suspected patients and populations at risk are difficult to implement in remote rural settings where the disease occurs. Availability of simple, easy to use, instrument-free rapid diagnostic tests would improve screening and coverage of the population at risk and contribute to elimination of the disease. It would enable technicians with limited training and clinicians in emergency or medical wards to make rapid differential diagnosis for neurological syndromes or malaria-like illnesses.

Introduction of such tests in all healthcare facilities in endemic regions would enable early detection of cases, hence reducing the time lost by patients before they get adequate and safe treatment. Treatment delay occurs when such patients attend non-specialized health centres that are unable to perform diagnosis of the disease. We evaluated a prototype rapid diagnostic test for HAT, the SD BIOLINE HAT in Angola, the Democratic Republic of the Congo and the Central African Republic. We show here that the test is as sensitive as the CATT in a 1/8 dilution and less sensitive than CATT on whole blood, although this latter difference was not statistically significant. The prototype RDT is a promising alternative for serodiagnosis of HAT.

Introduction

Human African trypanosomiasis (HAT) or sleeping sickness is a neglected tropical disease that is endemic in remote, resource-limited regions of sub-Saharan African countries [1]. The disease is transmitted by infected *Glossina* species (tsetse fly) and presents in two different forms caused by two different trypanosome subspecies, *Trypanosoma brucei (T. b.) gambiense* and *T. b. rhodesiense* respectively. The two disease forms differ in their geographic location, transmission pattern, clinical manifestation and response to treatment [2, 3]. More than 95% of reported cases of HAT are caused by *T. b. gambiense* which is the focus of this study. Both forms of HAT evolve in two disease phases: an early or haemolymphatic phase or stage one, and a late meningoencephalitic phase or stage two. During stage one, clinical signs are non-specific and can easily be confounded with a malaria-like illness, whereas neurological signs (sleep disorders, abnormal movements, gait disturbances and/or psychiatric disturbances) insidiously develop and characterize the stage two disease [4]. Control of *T. b. gambiense* HAT is dependent on diagnosis and treatment of infected individuals. Due to the chronic nature of the disease and a lack of specific symptoms during stage one, identification of cases relies on passive and active screening of patients and populations in endemic areas. Suspects identified using a screening test have to be confirmed as cases by demonstration of parasites in either blood, lymph node aspirates or the cerebrospinal fluid (CSF) by microscopy [4].

Thanks to international and national efforts, the number of cases of HAT reported to the World Health Organization (WHO) have been declining, from 37,991 in 1998 to 3,796 in 2014 [5,6,7]. Although the drop in cases is encouraging, there is concern that interest and funding of activities related to surveillance and control of the disease could go down, increasing the risk of resurgence [8]. In this context, and considering WHO's goal of eliminating HAT by 2020 that was endorsed by the London Declaration of 2012 [9], it is of particular importance to implement novel control strategies. Elimination of the disease and sustaining elimination will require improved surveillance and coverage of the population at risk, using tools that have a higher accuracy, and easier to deploy in the settings where the disease occurs. This can be hampered by challenges in diagnosis of the disease, as current screening algorithms are neither sensitive enough, nor easy to use in peripheral health centres where patients first seek care, or in general hospital wards. Indeed, infected people can spend a long time seeking appropriate care without a correct diagnosis being made, ending up in stage two [10]. Management of the disease when it has advanced into the second stage is complicated, expensive (due to the requirement for inpatient treatment), and associated with risks of sequelae and relapses. There is therefore a great need for accurate, easy to use and affordable diagnostic tests for HAT that can be used to screen individuals presenting with symptoms with a differential diagnosis that includes HAT [11].

Such tests would enable integration of HAT diagnosis in the general primary health care system, thus improving coverage of the population at risk.

Alere/Standard Diagnostics, Inc., South Korea, in collaboration with among others the Foundation for Innovative New Diagnostics (FIND), Switzerland and the Institute of Tropical Medicine (ITM) in Belgium, has developed a number of prototype rapid diagnostic tests (RDTs) for HAT using different trypanosome antigen combinations. Comparison of the prototype RDTs with the reference antibody detection screening test in current use, the card agglutination test for trypanosomiasis (CATT/*T.b.gambiense*) [12] using stored plasma samples showed that the SD BIOLINE HAT performed as well as CATT (S1 File). A limitation of that comparison however, was that the plasma samples had been pre-selected using CATT, as it was the only screening test for HAT that was in use at the time. The aim of this study was to evaluate the diagnostic accuracy of the prototype SD BIOLINE HAT using fresh blood samples under field conditions in a cross-sectional study; to compare its performance with that of CATT, on subjects who are not pre-selected using another serological method. CATT on 1/8 diluted plasma, which is more specific than CATT on whole blood and may be more suited for the target diagnostic settings, was considered for the main comparison. The study was carried out in three countries, in order to assess whether regional differences associated with parasite strains, level of endemicity or population genetic polymorphisms would interfere with test results. Angola, the Democratic Republic of the Congo (DRC) and the Central African Republic (CAR) were selected as countries with low (0.1–0.5%), intermediate (0.5–1.5%) and high (1.5–3.0%) prevalence, respectively. Subjects were enrolled through passive screening in fixed healthcare facilities and through active screening by mobile teams.

Methods

Participants

The settings, prevalence, names and locations of the study sites are summarized in Table 1. Study participants were enrolled in HAT endemic regions during both active and passive screening activities by teams of the national sleeping sickness control programs in Angola, the DRC and CAR. Cases of HAT were defined as subjects in whom trypanosomes were demonstrated by microscopy in either blood, lymph node aspirate or CSF.

3.1 Table 1. Sites in Angola, CAR and the DRC where participants were enrolled by both passive and active screening.

Prevalence was calculated on the basis of combined active and passive screening results from this study.

Country	County/ prefecture/ province	HAT prevalence	Commune/health district	Fixed centre	Mobile team
Angola	Bengo, Uuige	0.3%	Caxito	Caxito, Uige	Mumbondo
	Kwanza Norte		Mumbondo	Ndalatando	
CAR	Batangafo	2.13%	Hama, Bakassi		Batangafo
			Ouassi		
DRC	East Kasaï	1.2%	Bibanga, Tshilenge	Tshibila	Tshilenge

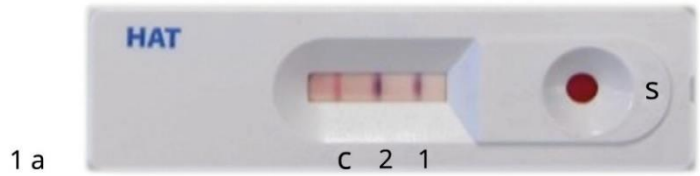
CAR = Central African Republic; DRC = Democratic Republic of the Congo.

Cases were classified as stage one when no trypanosomes were observed in CSF and when the CSF white cell count was lower than or equal to 5 cells/μL, while those with trypanosomes in CSF and/or a CSF cell count above 5 cells/μL were classified as stage two. Controls were subjects living in the same areas as cases, with no previous history of HAT treatment and who were either seronegative (negative with all serological tests), or who were seropositive (positive with one or more serological tests) but with no detectable parasites in body fluids.

Test procedures

The prototype SD BIOLINE HAT is an immunochromatographic test for qualitative detection of antibodies of all isotypes (IgG, IgA and IgM). The test has a nitrocellulose membrane strip with two regions (T1 and T2) that are pre-coated with two native variant surface glycoprotein (VSG) antigens from *T.b. gambiense* (VSG LiTat1.3 and VSG LiTat 1.5 respectively). It also has a procedural control line (C) (Fig 1). The test is stable for at least 24 months at 40°C, or at least 5 weeks at 55°C. To perform the test, 20 μl of whole blood is taken from a finger prick and transferred into a sample well using a plastic pipette, and 4 drops (approximately 120 μl) of chase buffer are added. The sample flows along the membrane by capillarity, passing through the two test regions. Results are read after 15 to 20 minutes by comparing the intensity of the test lines against a colour chart provided by the manufacturer (Fig 1a and 1b). A result is considered positive when the control line C and either one or both test lines T1 and T2 are visible, negative when only the C line is observed and invalid if the C line is not observed. All participants found positive with the RDT or CATT test were tested for malaria using an SD BIOLINE malaria Ag *P.f* RDT. Those who were positive for malaria were examined, and if necessary, treated in line with national guidelines.

Both CATT and SD BIOLINE HAT were performed on finger-prick blood from each subject who presented to mobile teams and any subject who presented to a health centre with symptoms indicative of HAT. Any subject who was positive by the RDT and/or CATT or who showed symptoms highly suggestive of HAT (i.e. a combination of at least 2 neurological signs) was eligible for enrolment in the parasitological work-up. Written informed consent was sought from these subjects prior to enrolment. Any individual who declined to participate was followed up according to the standard procedures of the national control programme. Individuals who were negative in both CATT and RDT were considered as not infected and were not investigated further. A lymph node aspirate was collected from any subject who presented with swollen lymph nodes and examined for trypanosomes by microscopy. Ten ml venous blood with heparin as anticoagulant, were collected from each RDT and/or CATT positive subject, as well as those with palpable lymph nodes. Six hundred µl of blood was used to perform the capillary tube centrifugation (CTC) test (4 capillary tubes of 75 µl) and the miniature anion exchange centrifugation technique (mAECT) (300 µl) [13], except in CAR where mAECT was not performed as it was not in routine use. For subjects who were positive by CATT on whole blood, 1 ml plasma was used to perform CATT dilutions [14]. Parasitologically confirmed cases and/or subjects found positive by CATT at a dilution of 1/16 who were negative by all other parasitological methods that were performed underwent a lumbar puncture in accordance with national guidelines for stage determination and/or parasitological confirmation in CSF when there were suggestive neurological signs. Parasitological examination of CSF was done using the modified single centrifugation technique [13]. The technicians who performed the tests were part of the teams of the national sleeping sickness control programmes, with experience in performing routine parasitological tests for detection of trypanosomes. They were trained on how to perform, read and interpret results of the RDTs.



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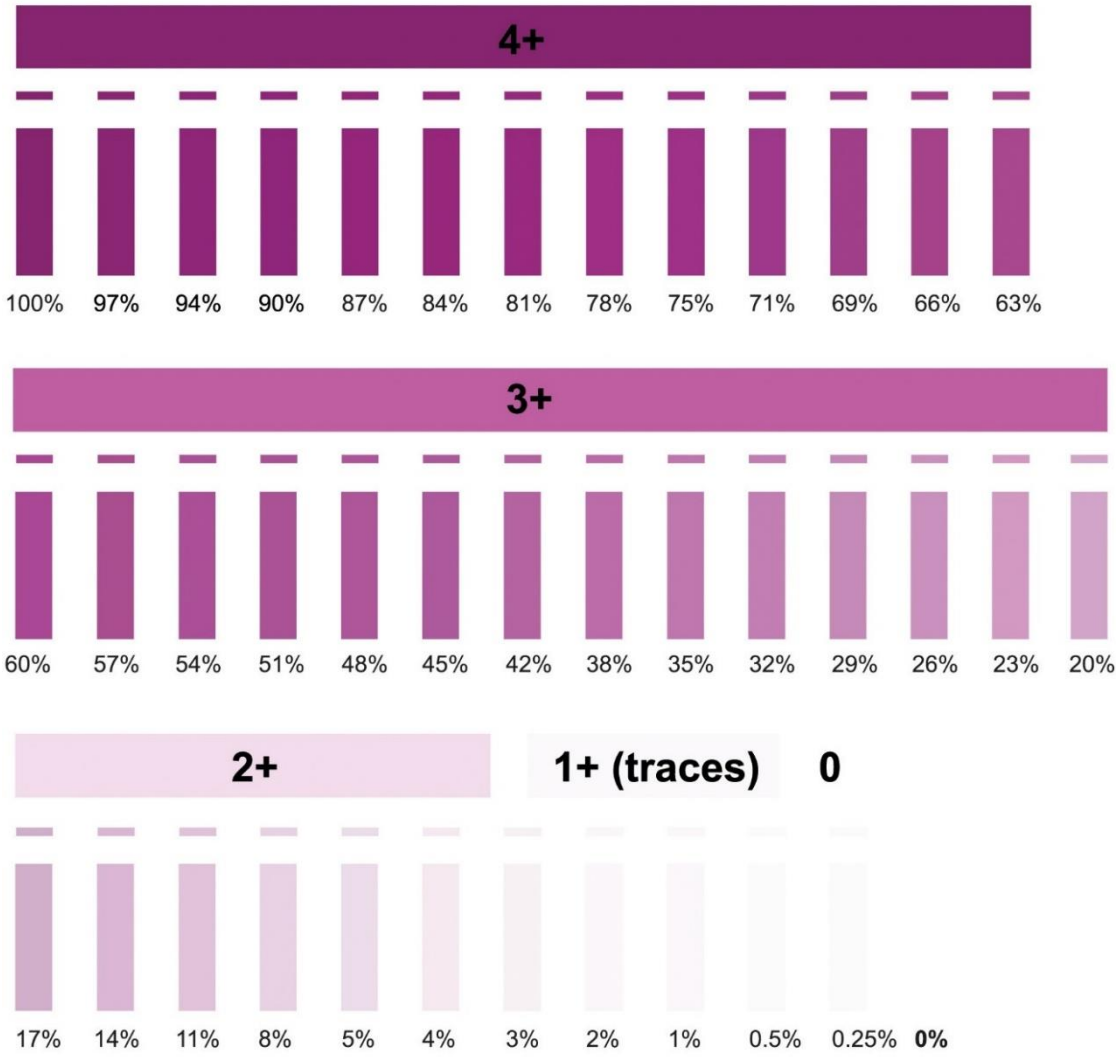

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3.1 Figure 1. Example of an SD BIOLINE HAT positive test and the colour chart provided for interpretation of test intensity

the color chart is for research purposes only, the final test would be as positive or negative result only). 1a: from right to left: S: sample well, 1: band 1(LiTat 1.3), band 2 (LiTat 1.5). 1b: from top to bottom: possible results 4+, 3+, 2+, 1+, 0, and the range of color intensity corresponding to each result.

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There were two levels of blinding. During the initial screening of participants using blood from a finger prick, one health worker was responsible for performing the CATT test, while another health worker tested them with the RDT. The two health workers operated independently (but used blood from the same finger prick), without exchanging results (first level of blinding). A supervisor was responsible for collecting results of the two tests and deciding whether or not to collect venous blood for the parasitological tests. Samples of venous blood were labelled with blinding codes by the supervisor (second level of blinding). The same codes were used to identify all other samples collected from the participants (e.g. the buffy coat, plasma, CSF and/or blood on filter paper) and constituted the anonymisation process kept all along the study.

All plasma, buffy coat and CSF samples that remained after the diagnostic procedures were aliquoted and stored in liquid nitrogen. Due to logistical constraints in CAR that limited access to liquid nitrogen for sample storage, blood was dried on Whatman filter paper, while plasma was stored at 4°C in the field for not more than 2 weeks before being transferred to Bangui, where it was stored at -20°C. The samples were later shipped to either Limoges University (France) or Makerere University (Uganda) for storage. Samples from a subset of subjects who were positive by any screening method but not by parasitology were later tested with two molecular methods, including the loop-mediated isothermal amplification (LAMP) developed by Eiken Chemical Co. [15] and polymerase chain reaction (PCR) using primers specific for the *T. brucei* group [16].

Sample size calculation

We aimed to enrol sufficient study participants to give a robust estimate of test performance parameters whilst ensuring that the target sample size would be logistically feasible for such a prospective study. In order to achieve this, we calculated a sample size to demonstrate non-inferiority of the sensitivity and specificity of the RDT compared to the CATT 1/8 with a confidence interval (1-alpha) of 95% and a power (1-beta) of 80%, a non-inferiority margin of 5%, and using an expected sensitivity and specificity of 95% for both tests. The minimum number of study participants required to achieve this was 235 true HAT cases and 235 controls [17].

Based on the expected prevalence of HAT in the study areas, the minimum number of subjects estimated to be enrolled in order to get at least 235 HAT cases were 6,320 in Angola and 4,200 in each of the DRC and CAR, for a total of 14,720 subjects.

Statistical analysis

Sensitivity and specificity were calculated for the prototype RDT, CATT on whole blood and CATT on 1/4 and 1/8 diluted plasma, by country, by disease stage and by screening method. In the field, CATT dilutions are sometimes performed on subjects who are negative by CATT on whole blood if the subject has symptoms indicative of HAT. However, since CATT dilutions are usually not performed on subjects who are negative by CATT on whole blood, we considered any subject who was negative by CATT on whole blood to also be negative by CATT on diluted plasma for purposes of the analysis.

The results of the serological tests CATT and RDT were each compared to a composite reference standard (CRS). This CRS classified participants based on demonstration of trypanosomes by any of the parasitological methods. A participant was classified as CRS positive, and hence considered as a HAT case, when parasites were demonstrated by any of the parasitological tests. A participant was classified as CRS negative if parasites were not identified in any body fluid, provided that at least CTC and mAECT had been performed (except in CAR where mAECT was not used).

The results on diagnostic accuracy of the tests are reported in a descriptive manner, without claiming non-inferiority for two reasons: the required sample size could not be reached as the prevalence of HAT was lower than expected and the actual sensitivity and specificity of the CATT 1/8 and the RDT were also lower than anticipated. Therefore, 95% confidence intervals (CI) were calculated around the sensitivity and specificity estimates using the Wilson method implemented in the Hmisc package for the R statistical environment [18, 19]. Statistical significance was analysed by checking for an overlap in confidence intervals and more formally using Pearson's Chi-squared test.

In an additional analysis, we compared the results of molecular analyses to those of parasitology to identify participants who were potentially parasitological false negatives.

Ethical approval

The study received ethical clearance from the different committees of the three participating countries respectively for DRC, CAR and Angola: "Ecole de santé publique de l'Université de Kinshasa"; "Comité scientifique de la faculté des sciences de la santé" and "direccao nacional da saude publica, Ministerio da saude". Participants provided written informed consent before being enrolled in the study. For children below 18 years, consent was provided by a parent or guardian. All individuals who presented at study sites during the period of enrollment and consented to being screened were eligible. All participants' samples were blinded and further analysed anonymously.

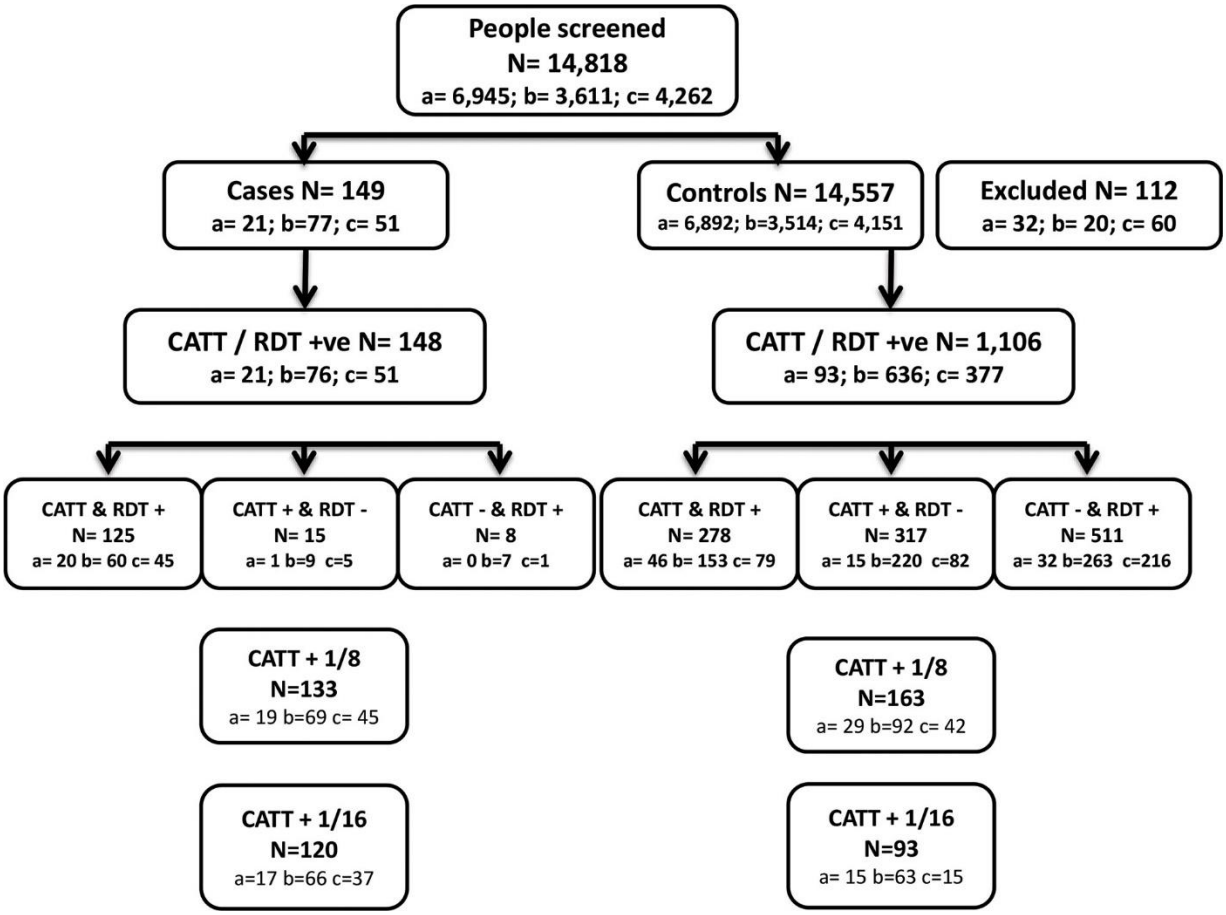
Results

Overview of participants and test results

Enrolment of participants was carried out from July to October 2011 in Angola, September 2012 to March 2013 in the DRC, and from April to June 2012 in CAR. The global and country-specific overview of the results of the study is shown as a flow chart in Fig 2. In total, 14,818 participants were screened using both serological tests (9.5% in passive screening), and out of these 149 HAT cases were confirmed by parasitology. Hence, the prevalence of HAT was found to be lower than originally estimated, and as such it was not possible to reach the target number of cases. Among the remaining 14,669 participants, 112 could not be included as controls

because 98 had previous history of HAT, and 14 who were positive by serology were excluded during analysis because their parasitology results were incomplete (CTC had not been performed).

Among the 149 confirmed cases, 148 were positive by either CATT on whole blood and/or RDT, while one case was negative by both CATT whole blood and RDT, and was detected by CATT dilutions. CATT dilutions were performed on this case because of the presence of symptoms suggestive of HAT. Twenty-one cases were identified in Angola (detection rate of 0.30%), 77 in CAR (detection rate of 2.13%) and 51 in the DRC (detection rate of 1.20%). The median age of cases was 24 years (range 1–78) with a sex ratio of 0.84 (68 males/81 females). One hundred and nine cases (73.2%) were enrolled during active screening and 40 (26.8%) during passive screening. Forty six (31%) cases were in stage one disease and 103 (69%) were in stage two. Forty percent of cases identified by active screening were in stage one disease, compared to only 5% among cases identified by passive screening.



3.2 Figure 2. Overview of the results of the CATT and RDT tests in Angola, CAR and the DRC. RDT: SD BIOLINE HAT rapid diagnostic test. CATT : card agglutination test for trypanosomiasis. * 1 case that was negative on CATT whole blood and the RDT was detected by CATT 1/8 dilution. ** a = Angola; b = Central African Republic; c = Democratic Republic of the Congo. / = and/or
doi:10.1371/journal.pntd.0004608.g002

Among the 14,557 controls, 1,106 (7.6%) were positive with either one or both screening tests, and 13,437 were negative with both. Of these controls, 6,892 (47.4%) were enrolled in Angola (5,877 by active screening and 1,013 by passive screening), 3,514 (24.2%) in CAR (all by active screening) and 4,151 (28.5%) in the DRC (3,816 by active screening and 335 by passive screening). Among the 1,106 positive tests, 278 were positive with both serological tests, 317 were positive by CATT and negative by RDT and 511 were negative by CATT and positive by RDT. No invalid RDT result was reported from any country.

Eight hundred and twenty-seven subjects (548 seropositive with at least one test and 279 positive by both tests) were tested using LAMP and PCR. Both molecular tests were negative in 664 (80.29%) samples, 65 (7.86%) were positive with LAMP only and 72 (8.71%) with PCR only. Both molecular tests were positive in 26 (3.14%) samples, 25 of which were from CAR where mAECT had not been used for parasitological confirmation of cases.

Results on sensitivity and specificity for each test and each CATT dilution by country, screening method and disease stage are shown in Tables 2, 3 and 4 respectively.

The overall sensitivity of the RDT and CATT at 1/8 dilution was identical, at 89.26% (95% CI = 83.27– 93.28), while that for CATT on whole blood was higher, at 93.96% (95% CI = 88.92–96.79%). Sensitivity of CATT on whole blood was highest in Angola and lowest in CAR. However, in CAR CATT retained its sensitivity when serial dilutions were considered, whilst in Angola and DRC the sensitivity declined, such that a dilution of 1/16 had the highest sensitivity in CAR (Table 2). The sensitivity of the RDT was highest in Angola and lowest in CAR. It is noticeable that the sensitivity of band 1 on the RDT, which has the same antigen as the one in the CATT test, was very similar in the three countries (85.71; 85.71 and 86.27% respectively for Angola, CAR and DRC), whilst the sensitivity of band 2 varied from 95.24% in Angola (95% CI = 77.33–99.76) to 83.12% (95% CI = 73.23–89.86) in CAR.

The overall specificity was 94.58% (95% CI = 94.20–94.94%) for the RDT, 95.91% (95% CI = 95.58–96.22%) for CATT on whole blood, and 98.88% (95% CI = 98.70–99.04%) for CATT at 1/8 dilution. There were significant differences ($p<0.001$) between the three countries in the specificity of all tests except for CATT 1/16 (99.36% overall specificity (95% CI = 99.22– 99.48%) (Table 2). As was seen with sensitivity, all tests were most specific in Angola and least specific in CAR.

Both the RDT and CATT on whole blood were more sensitive in stage two than in stage one patients (Table 4). Similarly, both the RDT and CATT on whole blood were more sensitive in passive than in active screening (Table 3). However, when CATT at 1/8 dilution was considered, sensitivity was higher in active than in passive screening (Table 3). The specificity of all tests was higher in passive than active screening (Table 3).

If a hypothetical cohort of 1,000 subjects with a HAT prevalence of 1% is considered, then the RDT would result in 53 false positives proceeding for confirmatory testing, while CATT WB and CATT 1/8 would results in 40 and 11 false positives respectively (Table 5).

Agreement between CATT on whole blood and RDT was 93.91%, with both positive for 426 (2.87%) participants (Table 6). Both tests were negative in 13,489 (91%), 361 (2.4%) were CATT positive and RDT negative, while 542 (3.6%) were CATT negative and RDT positive. Agreement increased slightly when band 1 of the RDT was considered on its own (94.53%) but was similar for RDT band 2 (93.99%).

Discussion

This study is the first report of a multi-centric evaluation of an RDT for screening HAT in settings where the disease occurs in central African countries. It is the first study conducted without pre-selection of participants on the basis of their CATT status, the only screening test that was in routine used at that time. The overall sensitivity of the SD BIOLINE HAT RDT and CATT at 1/8 dilution were identical (89.26%, 95% CI 83.27,

93.28%). The RDT was less sensitive than CATT on whole blood, although this difference was not statistically significant. However, the specificity of the RDT was 4.3% lower than that of CATT at 1/8 dilution, at 94.58% (95% CI 94.20, 94.94%). Comparison of the prototype RDT to CATT at 1/8 dilution was in line with the knowledge that the specificity of CATT is improved when this dilution is considered.

The sensitivity of the prototype RDT (89.26%) was lower than that reported in a recent study in the DRC on another RDT (98.5% for the HAT Sero-*K*-SeT) [20]. This may be explained by methodological differences, as our study included both active and passive screening, none of the study participants had been pre-selected with CATT, and the level of blinding was higher.

3.2 Table 2. Performance of CATT and prototype SD BIOLINE HAT RDT on fresh blood by country

	NB OF HAT CASES TESTING +VE	SENSITIVITY (95% CI)	NB OF CONTROLS TESTING -VE	SPECIfiCITY (95% CI)
CATT WB				
ALL	140 \ 149	93.96 (88.92–96.79)	13,962 \ 14,557*	95.91 (95.58–96.22)
ANGOLA	21 \ 21	100.0 (84.54–100.0)	6,831 \ 6,892	99.11 (98.86–99.31)
CAR	69 \ 77	89.61 (80.82–94.64)	3,141 \ 3,514	89.39 (88.32–90.36)
DRC	50 \ 51	98.04 (89.70–99.90)	3,990 \ 4,151	96.12 (95.49–96.67)
CATT 1/8				
ALL	133 \ 149	89.26 (83.27–93.28)	14,388 \ 14,551*	98.88 (98.7–99.04)
ANGOLA	19 \ 21	90.48 (71.09–97.35)	6,863 \ 6,892	99.58 (99.4–99.71)
CAR	69 \ 77	89.61 (80.82–94.64)	3,419 \ 3,511	97.38 (96.8–97.86)
DRC	45 \ 51	88.24 (76.62–94.49)	4,106 \ 4,148	98.99 (98.63–99.25)
CATT 1/16				
ALL	120 \ 149	80.54 (73.45–86.09)	14,458 \ 14,551*	99.36 (99.22–99.48)
ANGOLA	17 \ 21	80.95 (60.00–92.33)	6,877 \ 6,892	99.78 (99.64–99.87)
CAR	66 \ 77	85.71 (76.2–91.83)	3,448 \ 3,511	98.21 (97.71–98.59)
DRC	37 \ 51	72.55 (59.05–82.89)	4,133 \ 4,148	99.64 (99.40–99.78)
RDT ALL BANDS				
ALL	133 \ 149	89.26 (83.27–93.28)	13,768 \ 14,557	94.58 (94.2–94.94)
ANGOLA	20 \ 21	95.24 (77.33–99.76)	6,814 \ 6,892	98.87 (98.59–99.09)
CAR	67 \ 77	87.01 (77.72–92.79)	3,098 \ 3,514	88.16 (87.05–89.19)
DRC	46 \ 51	90.20 (79.02–95.74)	3,856 \ 4,151	92.89 (92.07–93.64)
RDT BAND 1				
ALL	128 \ 149	85.91 (79.41–90.59)	13,910 \ 14,557	95.56 (95.21–95.88)
ANGOLA	18 \ 21	85.71 (65.36–95.02)	6,829 \ 6,892	99.09 (98.83–99.28)
CAR	66 \ 77	85.71 (76.20–91.83)	3,116 \ 3,514	88.67 (87.58–89.68)
DRC	44 \ 51	86.27 (74.28–93.19)	3,965 \ 4,151	95.52 (94.85–96.11)
RDT BAND 2				
ALL	128 \ 149	85.91 (79.41–90.59)	13,839 \ 14,557	95.07 (94.7–95.41)
ANGOLA	20 \ 21	95.24 (77.33–99.76)	6,818 \ 6,892	98.93 (98.65–99.14)
CAR	64 \ 77	83.12 (73.23–89.86)	3,129 \ 3,514	89.04 (87.97–90.03)
DRC	44 \ 51	86.27 (74.28–93.19)	3,892 \ 4,151	93.76 (92.98–94.46)

*Number of controls for CATT dilutions are less by 6 (14,557 versus 14,551) because these were positive by whole blood, but no dilutions were performed.

RDT: SD BIOLINE HAT rapid diagnostic test; CATT: card agglutination test for trypanosomiasis.

WB: whole blood; 1/8, 1/16 are dilutions for the CATT test; CAR: Central African Republic; DRC: Democratic Republic of the Congo.

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During passive screening, a patient is subjected to a screening test after being suspected of having HAT, while in active screening, all people who present themselves are screened. As a result of the clinical suspicion before being tested, a passive screening setting may therefore bias the results towards higher sensitivity. The two studies are however in agreement that a combination of at least two antigens (VSG LiTat 1.3 and VSG LiTat 1.5) on the same test is necessary to provide sufficient sensitivity. In our study, using a single antigen would have resulted in a drop of 3.3% in sensitivity, whilst the specificity would have increased by less than 1%. In

another study that compared the diagnostic accuracy of the commercialized versions of both RDTs on stored serum samples from West Africa, the sensitivity of both tests was reported to be very good while the specificity was low [21]. This might be explained by geographic differences in the specificity of responses to VSG antigens but it may also suggest that results obtained using stored samples could differ from those obtained on fresh whole blood samples or alternatively, some changes in the buffer to optimize sensitivity could also modify specificity.

3.3 Table 3. Performance of CATT and prototype SD BIOLINE HAT RDT on fresh blood by method of screening

	NB OF HAT CASES TESTING +VE	SENSITIVITY (95% CI)	NB OF CONTROLS TESTING -VE	SPECIfICITY (95% CI)
CATT WB				
ALL	140 \ 149	93.96 (88.92–96.79)	13,962 \ 14,557	95.91 (95.57–96.22)
ACTIVE	40 \ 40	91.74 (85.05–95.60)	12,635 \ 13,209	95.65 (95.29–95.99)
PASSIVE	69 \ 77	100.0 (91.24–100.0)	1,327 \ 1,348	98.44 (97.63–98.98)
CATT 1/8				
ALL	133 \ 149	89.26 (83.27–93.28)	14,388 \ 14,551	98.88 (98.7–99.04)
ACTIVE	99 \ 109	90.83 (83.93–94.94)	13,048 \ 13,203	98.83 (98.63–99.00)
PASSIVE	34 \ 40	85.00 (70.93–92.94)	1,340 \ 1,348	99.41 (98.83–99.70)
CATT 1/16				
ALL	120 \ 149	80.54 (73.45–86.09)	14,458 \ 14,551	99.36 (99.22–99.48)
ACTIVE	88 \ 109	80.73 (72.34–87.04)	13,115 \ 13,203	99.33 (99.18–99.46)
PASSIVE	32 \ 40	80.00 (65.24–89.50)	1,343 \ 1,348	99.63 (99.13–99.84)
RDT ALL BANDS				
ALL	133 \ 149	89.26 (83.27–93.28)	13,768 \ 14,557	94.58 (94.2–94.94)
ACTIVE	96 \ 109	88.07 (80.66–92.90)	12,450 \ 13,209	94.25 (93.84–94.64)
PASSIVE	37 \ 40	92.50 (80.14–97.42)	1,318 \ 1,348	97.77 (96.84–98.44)
RDT BAND 1				
ALL	128 \ 149	85.91 (79.41–90.59)	13,910 \ 14,557	95.56 (95.21–95.88)
ACTIVE	94 \ 109	86.24 (78.53–91.48)	12,584 \ 13,209	95.27 (94.89–95.62)
PASSIVE	34 \ 40	85.00 (70.93–92.94)	1,326 \ 1,348	98.37 (97.54–98.92)
RDT BAND 2				
ALL	128 \ 149	85.91 (79.41–90.59)	13,839 \ 14,557	95.07 (94.7–95.41)
ACTIVE	91 \ 109	83.49 (75.40–89.29)	12,516 \ 13,209	94.75 (94.36–95.12)
PASSIVE	37 \ 40	92.50 (80.14–97.42)	1,323 \ 1,348	98.15 (97.28–98.74)

RDT: SD BIOLINE HAT rapid diagnostic test; CATT: card agglutination test for trypanosomiasis.
WB: whole blood; 1/4, 1/8, 1/16 are dilutions for the CATT test.

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In the present study, 16 cases would have been missed if only the prototype RDT had been used, and 9 if only CATT on whole blood had been used, reflecting the imperfect nature of both tests. Based on these observations, further optimization of the prototype RDT to improve both its sensitivity and specificity, which is critical when a test has to be used in situations of low prevalence, is recommended. The one case that was missed by CATT and RDT but found positive by CATT on diluted plasma could indicate the existence of a prozone effect, as has been reported with other serological tests [22].

Both of the antigens used in the prototype RDT are produced from pathogenic trypanosomes harvested from artificially infected rodents. Difficulties in their production could pose a challenge in scaling up production of the test. Altogether, both tests are imperfect, and reflect the difficulty of their use in settings of low prevalence and/or insufficient knowledge on parasite strain variation and/or the elicited immune reactions, highlighting the need for more investments in development of optimal tests. Recently, promising results were reported when prototype RDTs made using recombinant antigens were compared with the commercialized version of the SD BIOLINE HAT [23].

3.4 Table 4. Sensitivity of CATT and prototype SD BIOLINE HAT RDT on fresh blood by stage of disease

	Nb of patients testing +ve	**Sensitivity (95% CI)**
CATT WB		
All	**140 \ 149**	**93.96 (88.92–96.79)**
Stage 1	42 \ 46	91.30 (79.68–96.57)
Stage 2	98 \ 103	95.15 (89.14–97.91)
CATT 1/8		
All	**133 \ 149**	**89.26 (83.27–93.28)**
Stage 1	41 \ 46	89.13 (76.96–95.27)
Stage 2	92 \ 103	89.32 (81.88–93.93)
CATT 1/16		
All	**120 \ 149**	**80.54 (73.45–86.09)**
Stage 1	36 \ 46	78.26 (64.43–87.74)
Stage 2	84 \ 103	81.55 (72.98–87.86)
RDT all bands		
All	**133 \ 149**	**89.26 (83.27–93.28)**
Stage 1	38 \ 46	82.61 (69.28–90.91)
Stage 2	95 \ 103	92.23 (85.42–96.01)
RDT band 1		
All	**128 \ 149**	**85.91 (79.41–90.59)**
Stage 1	37 \ 46	80.43 (66.83–89.35)
Stage 2	91 \ 103	88.35 (80.73–93.21)
RDT band 2		
All	**128 \ 149**	**85.91 (79.41–90.59)**
Stage 1	35 \ 46	76.09 (62.06–86.09)
Stage 2	93 \ 103	90.29 (83.04–94.64)

RDT: SD BIOLINE HAT rapid diagnostic test; CATT: card agglutination test for trypanosomiasis. WB: whole blood; 1/8, 1/16 are dilutions for the CATT test.

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3.5 Table 5. Test outcomes based on a hypothetical cohort of 1,000 subjects with a HAT prevalence of 1%

	Infected	Non-infected
CATT WB		
Pos	9.4	40.47
Neg	0.6	949.53
CATT 1/8		
Pos	8.93	11.49
Neg	1.07	978.51
RDT		
Pos	8.93	53.66
Neg	1.07	936.34

RDT: SD BIOLINE HAT rapid diagnostic test; CATT: card agglutination test for trypanosomiasis; Neg: negative test, Pos: positive test.

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3.6 Table 6. Agreement between CATT on whole blood and SD BIOLINE HAT RDT, RDT band 1 and RDT band 2. This table includes all enrolled participants.

	CATT		Non-infected
	Neg	Pos	
RDT			
Neg	13,489	361	93.91
Pos	542	426	
RDT band 1			
Neg	13,615	396	94.53
Pos	415	392	
Neg	1.07		978.51
RDT			89.13 (76.96–95.27)
Pos	8.93		53.66
Neg	1.07		936.34

RDT: SD BIOLINE HAT rapid diagnostic test; CATT: card agglutination test for trypanosomiasis; Neg: negative test, Pos: positive test.

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This study had a number of limitations. Firstly, the study lacked the level of statistical power that we had aimed for, and secondly, the results of the present study were not systematically validated with reference tests for antibody detection such as immune trypanolysis [24]. Thirdly, the mAECT test, which is the most sensitive parasitological method for *T. b. gambiense* HAT [25], was not routinely used in the CAR and was therefore not included in that part of the study, potentially leading to the misclassification of some infected individuals as controls. It is possible that participants who were reported as false positives with either serological test were in fact true positives, compromising in particular the reported specificity of the screening tests. Indeed both tests were least specific in the CAR. This is supported by the observation that when LAMP and PCR were performed on 70% of the seropositive but parasitologically negative samples, 3.14% were positive on both tests (S2 File). Most of the positive samples were from CAR where mAECT, was not performed. Approximately 8% of seropositive samples were positive by either LAMP or PCR, pointing to the possibility that these could have been true infections that were missed by the methods that are currently in clinical use. Indeed recent retrospective studies using samples from HAT patient have shown that both methods, which detect parasite DNA, have a sensitivity of up to 90% [25, 26, 28]. However, when human-infective parasite DNA is detected, it is unclear whether this should be taken as a marker of infection or disease; both spontaneous resolution and persistent latent infection can occur [21, 26]. Introduction of such molecular tests into clinical use may be a challenge and strategies on how they could be used to contribute to elimination of the disease have still to be fully explored [21, 26, 27, 28].

RDTs have a number of advantages, especially in settings of low prevalence and where the target is to eliminate the disease. They are simple, instrument-free, easy to use, and do not require a cold chain for storage. This study has demonstrated that performance of the SD BIOLINE HAT RDT is comparable to that of CATT 1/8. The recommendations arising from the study have since been used to optimize the prototype RDT, and performance and implementation studies using the optimized product are going on in several countries.

Introduction of HAT RDTs in routine use as an alternative to CATT could have significant cost implications on the healthcare system, which should be determined and the information used to guide policy decisions. While the benefits of using RDTs for passive screening in health facilities are evident, the cost-effectiveness of algorithms that use RDTs in active screening in place of CATT should be evaluated. During active screening using CATT for example, blood samples from 10 individuals are tested in parallel, which is advantageous because a large number of people can be tested in one day. When RDTs are used instead, one person is tested at a time, and the results are read after 15 minutes, meaning that fewer people are likely to be screened in one day, with a negative impact on population coverage. Similarly, the reagents used to perform the CATT test are packed in multiple doses while RDTs are single devices, which occupy a larger space during transportation,

thus posing a challenge and additional costs to the mobile team. A detailed analysis of these aspects is currently being prepared as a follow on publication.

Supporting Information

S1 File. Comparison of the performance of antigens in a prototype HAT rapid diagnostic test (RDT) with CATT using stored samples.

(DOCX)

S2 File. Proportion of positive and negative test results using molecular analysis (LAMP and PCR).

(DOCX)

S3 File. STARD checklist.

(DOC)

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Author Contributions

Conceived and designed the experiments: SBis SBie JMN CL. Performed the experiments: SBis GV TJ CL VK EN LF. Analyzed the data: PRB. Contributed reagents/materials/analysis tools: PB. Wrote the paper: SBis PRB SBie JMN MB PB.

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3. Performance of the SD BIOLINE® HAT rapid test in various diagnostic algorithms for *gambiense* human African trypanosomiasis in the Democratic Republic of the Congo

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Abstract

We carried out a study to compare the performance, in terms of sensitivity and specificity, of the new SD BIOLINE® HAT rapid diagnostic test (RDT) with the card agglutination test for trypanosomiasis (CATT) for diagnosis of human African trypanosomiasis (HAT) in the Democratic Republic of the Congo (DRC). Participants were enrolled actively by four mobile teams, and passively at four health facilities in three provinces. Consenting participants were tested concurrently with the RDT and CATT on whole blood. Those found positive by either test were tested with CATT on serial dilutions of plasma, and with a parasitological composite reference standard (CRS). Cases were only the individuals found positive by the CRS, while controls were negative by both CATT and RDT, as well as those that were positive by CATT or RDT, but were negative by the CRS, and had no history of HAT. Over five months, 131 cases and 13,527 controls were enrolled. The sensitivity of the RDT was 92.0% (95% confidence interval (CI) = 86.1–95.5), which was significantly higher than CATT (sensitivity 69.1%; 95% CI = 60.7–76.4). The sensitivity of CATT on plasma at a dilution of 1:8 was 59.0% (95% CI = 50.2–67.2). The specificity of the RDT was 97.1% (95% CIs = 96.8–97.4) while that of CATT was 98.0% (95% CIs = 97.8, 98.2) and specificities of algorithms involving CATT at 1:8 dilution were 99.6% (95% CI = 99.5–99.7). Reproducibility of results was excellent. We concluded that an algorithm in which the SD BIOLINE® HAT RDT is used for screening is optimal for case detection in both passive and active screening settings. However, the lower specificity of the RDT compared to that of CATT would result in a larger number of false positive individuals undergoing confirmatory testing.

Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a vector-borne parasitic disease transmitted to humans by the bite of an infected tsetse fly (*Glossina spp*). The disease is endemic in sub-Saharan Africa, within the limits of the geographic distribution of the tsetse fly. Two sub-species of the protozoan parasite *Trypanosoma brucei* cause the disease in humans: *T.b. gambiense* and *T.b. rhodesiense*. Infection with *T.b. gambiense* causes the chronic form of HAT (*gambiense* HAT) and accounts for more than 95% of cases [1]. *Gambiense* HAT is endemic in rural, resource-limited settings, mainly in west and central Africa, with the majority of cases reported in the Democratic Republic of the Congo (DRC) [2]. The number of cases of HAT reported globally has been falling steadily, and the disease is now targeted for elimination as a public health problem by 2020 [3].

Control of *gambiense* HAT is based on detection and treatment of infected individuals and early and accurate diagnosis of the disease is essential, as early treatment is easier, safer, and more effective [4,5], while early detection truncates the window for onward transmission.

However, the early stage of *gambiense* HAT is often sub-clinical and once clinical signs appear, they are similar to those of malaria, a disease that is endemic in all regions where HAT occurs. As a consequence, it is necessary to screen a large number of people among the population at risk in order to identify HAT cases. The card agglutination test for trypanosomiasis (CATT) is at present the most commonly used test for screening HAT [6]. CATT is a serological test that detects host antibodies to infection, with a sensitivity of around 90% and specificity of between 97–99% [1,7–9]. The specificity of the test can be improved if it is repeated on serially diluted plasma from individuals who are positive on whole blood [10]. People who are positive by the screening test are submitted to confirmatory tests that are based on visualisation of trypanosomes by microscopy in lymph node aspirates, blood or cerebrospinal fluid (CSF). Due to the relatively low density of parasites in the blood of HAT cases, concentration techniques such as the micro-haematocrit centrifugation technique (mHCT or Woo test) and the mini-anion exchange centrifugation technique (mAECT) are used to enhance sensitivity by improving the likelihood that parasites will be visualised [11]. Following confirmation, the patient is treated according to the stage of disease, which is determined by performing a lumbar puncture and examining the CSF for the presence of trypanosomes, and counting the number of white blood cells (WBC) [12–14]. During early or stage 1 of the disease, parasites are found only in the haemolymphatic system, while the advanced or stage 2 disease is associated with presence of parasites in the CSF and/or more than 5 WBCs per µl [4].

While CATT has been widely used by vehicle- and boat-based mobile teams, including in the DRC [12], its use for passive screening is associated with a number of constraints. These include the requirement for a source of power and a cold-chain, and the 50 dose format of CATT means that when the reagent is reconstituted, the doses must be used within a few days in order to avoid spoilage [15,16]. To reduce this wastage, the Institute

of Tropical Medicine (ITM) developed the CATT D10, which contains reagents for 10 tests [16,17]. Whilst the CATT D10 is thermostable, the vial of reagent must be used within 24 hours after opening, which has restricted its widespread use by national HAT control programmes. As an alternative to CATT, two rapid diagnostic tests (RDTs) have recently been developed and commercialized. These are the SD BIOLINE® HAT RDT developed by Alere/Standard Diagnostics, Inc. (SD) in collaboration with the Foundation for Innovative New Diagnostics (FIND) [18], and the HAT Sero-K-Set developed by Coris BioConcept in collaboration with the ITM [19,20]. Both are first generation RDTs based on native antigens, and at the time of writing, were the only HAT RDTs that have been commercialised. RDTs are performed on fresh blood from a finger prick, do not require any instrument, and test results are obtained in 15 minutes.

With these characteristics, RDTs could play a major role in both screening for HAT at health facilities at the lowest level of the healthcare system, and in active screening by health workers at the level of the community.

The SD BIOLINE® HAT RDT is stable for at least 24 months at 40˚C, and detects antibodies against two trypanosome variable surface glycoprotein (VSG) antigens (LiTat 1.3 and LiTat 1.5) which are incorporated as separate bands. When a test is performed and any of the test bands is observed, the result is interpreted as positive. The CATT on the other hand detects antibodies against *T. b. gambiense* parasites expressing VSG LiTat 1.3. A prototype of the SD BIOLINE® HAT RDT was found to have a sensitivity of 89.3% and a specificity of 94.6%, but its performance varied by geographic settings [21]. The sensitivity was not significantly different to that of CATT on whole blood ($p = 0.21$) or CATT on plasma at a dilution of 1:8 ($p = 0.85$). The specificity of the prototype RDT was 94.6%, which was significantly lower ($p<0.001$) than that of CATT on whole blood (95.9%), and significantly lower ($p<0.001$) than the specificity of CATT on plasma at a dilution of 1:8 (98.9%) [21]. Based on these results, the SD BIOLINE® HAT RDT was optimized further to improve sensitivity, without compromising specificity. This was done at the level of manufacturing, by changing the composition of buffer that is used in the test, and validated by testing stored samples from 49 HAT cases and 399 HAT negative controls.

This study was carried out to evaluate the performance (sensitivity, specificity and reproducibility) of the SD BIOLINE® HAT RDT in field settings in the DRC, by comparing the RDT and CATT as the screening test in both active (by mobile teams) and passive (in health facilities) settings. This was part of a large study to demonstrate the use of the HAT RDT as part of the routine screening activities of the national HAT control programme of the DRC.

Materials and methods

Study sites

This study was carried out in the provinces of Bandundu (Kwamouth and Bagata general hospitals, Kwamouth and Bagata mobile teams; now in Mai-Ndombe and Kwilu provinces), East Kasaï (Lukalaba hospital and Miabi mobile team) and West Kasaï (Kakenge reference health centre and Kakenge mobile team, now Kasai province) in the DRC. Study sites were visited by an external monitor prior to commencement of the study to verify that they were adequately prepared and staff were properly trained, and during the study, they were visited to ensure that the protocol was being adhered to.

Enrolment of participants

Participants were enrolled passively in the 4 health facilities, and actively by the 4 mobile teams. In health facilities, participants were enrolled from among patients presenting themselves or referred from other health facilities after suspicion of HAT or other diseases, and among relatives who accompanied patients. During active screening, all those who presented themselves to the mobile team were eligible for enrolment in the study. From those found positive by the RDT or CATT, written informed consent was sought. No additional information or samples were collected from those that were negative by RDT and CATT; only a count of the numbers screened for use in specificity analysis, and hence there was no requirement for informed consent. People who presented for screening but did not wish to participate in the study were screened according to the procedures of the national programme. All consented participants were tested for malaria and if found positive, they were managed according to the guidelines of the national malaria control programme but remained eligible for enrolment in this study.

Tests performed

The CATT and HAT RDT were performed on fresh blood obtained from a finger prick for each individual presenting for active or passive screening. The results of CATT and RDT were each read independently by two laboratory technicians or nurses who were specialized in HAT, and the results were recorded separately. The technicians were blinded to the result of one another. For the RDT, the overall result of the RDT was recorded as per the manufacturer's guidelines. A positive result is a reaction on either of the two bands corresponding to VSG LiTat 1.3 (band 1) or VSG LiTat 1.5 (band 2), whilst a reaction on the control band alone is a negative result. The intensity of each RDT band was qualitatively assessed and scored from 0 to 4 according to a printed scale that was provided by the manufacturer, with 0 equating to absence of a band. The overall result and the result on individual bands were recorded separately. The result of CATT was recorded simply as positive or negative, but a note was made for any CATT result whose interpretation was deemed to be a doubtful result.

Participants found positive by either reader by CATT or the RDT were tested with a parasitological composite reference standard (CRS), which involved carrying out a number of parasitological tests that are in standard use in the DRC. To perform the CRS, 5 ml venous blood was collected, and patients with palpable cervical lymph nodes had a lymph node aspirate taken and examined for motile parasites by bright field microscopy. If lymph node palpation was not possible or was negative, 500 µl of blood was used to perform the mini anion exchange centrifugation technique (mAECT) and 4 capillary tubes of 75 µl each were used for the microhaematocrit centrifugation technique (mHCT or Woo test) (Study protocol S2 File). The technician performing parasitology was blinded to the results of CATT and RDT, and any samples that were positive by a parasitological technique were verified by the unit's supervisor.

If the subject was positive by CATT, the remaining blood was centrifuged and 30 µl of plasma used to prepare dilutions for repeat testing with CATT. The results of CATT dilutions were also read by two technicians independently, and recorded separately.

If an individual was positive by CATT at 1:8 dilution or had symptoms strongly suggestive of HAT, but was negative by examination of lymph node aspirate, mHCT and mAECT, a lumbar puncture may have been performed and the CSF examined for trypanosomes by microscopy, which was at the discretion of the supervisor (Fig 1). Any patients with parasites or more than 5 WBCs per µl in the CSF were interpreted as stage 2 cases. All confirmed HAT cases were treated according to the guidelines of the national programme.

Data analysis

The definition of a HAT case was a study participant from whom trypanosomes were visualised in any fluid, including a lymph node aspirate, blood or CSF. A control was defined as either an individual found to be both RDT and CATT negative and who had no history of HAT (not treated for HAT in the past), or an individual who was either RDT and/or CATT positive but from whom no trypanosomes were seen in any body fluid (using at least both mHCT and mAECT), and had no history of HAT.

We assessed the sensitivity and specificity of three tests:

- Screening with CATT on whole blood,
- Screening with CATT on whole blood followed by CATT at 1:8 dilution.
- Screening with RDT.

The reference standard was parasitological confirmation using the CRS, and confirmed cases were staged by examination of the CSF.

The results obtained from fixed health facilities and from mobile teams were analysed separately. This is because these are two very different settings–mobile teams operate outdoors and screen all persons that present,

whilst fixed facilities only screen clinical suspects who present themselves and high risk individuals. In the analysis, we present the overall RDT result and the results of the individual bands.

Statistical analysis

Sensitivity was calculated as the number of cases that were positive by a screening test, divided by the total number of cases. Specificity was calculated as the number of controls that were negative by a screening test, divided by the total number of controls. As each screening test result was read by two people, the final number of positive results was the average result from the two readers, and in the event of discordant results, this was included as a 0.5 in the numerator for sensitivity and specificity calculations as per [18]. Results from parasitology were only recorded once, the single result that was confirmed by the supervisor. Patients that were positive by a screening test and were not positive by any parasitological method and had not completed the minimum CRS of mHCT and mAECT were excluded. 95% confidence intervals (CIs) were calculated using the Wilson method implemented in the Hmisc package [22] in the R statistical environment [23]. To test agreement between readers, we calculated Cohen's Kappa using the fmsb package [24] in R, we interpreted a Kappa of greater than 0.8 as very strong agreement [25] and used the *p*-value to evaluate whether it is significantly different from zero.

To interpret the impact of test sensitivity and specificity in terms of false positives and false negatives in consideration of the disease prevalence, we calculated the positive predictive value (PPV) as follows:

$$PPV = \frac{(sensitivity\ x\ prevalence)}{sensitivity\ x\ prevalence + (1 - \ specificity)\ x\ (1 - prevalence)}$$

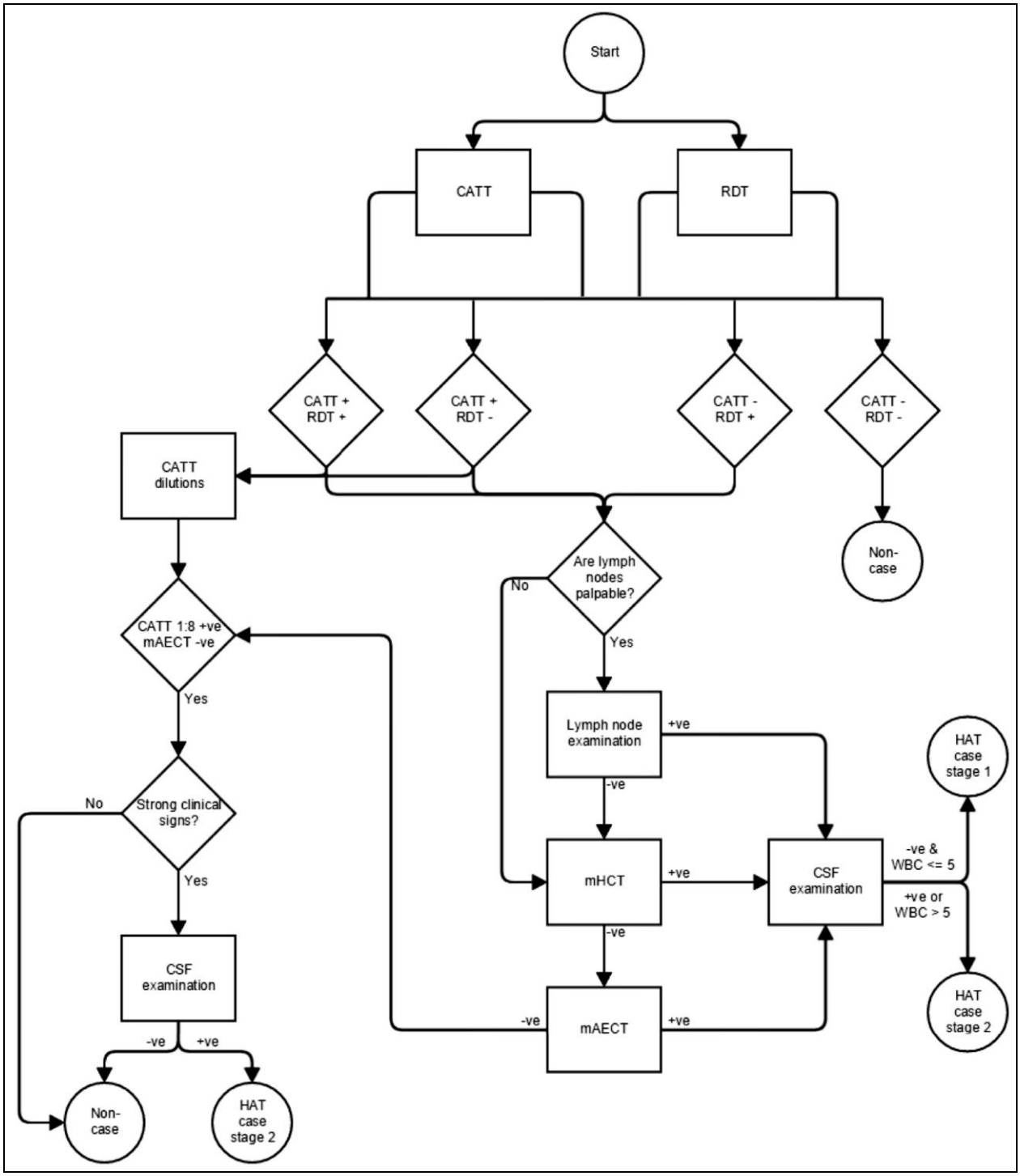
and the negative predictive value (NPV) as follows:

$$NPV = \frac{specificity\ x\ (1 - \ prevalence)}{(1 - sensitivity)\ x\ prevalence + \ specificity\ x\ (1 - prevalence)}$$

To make this a statistic that can be easily translated by a surveillance programme, we present the false discovery rate (FDR) and false omission rate (FOR) as:

$FDR = 1 - PPV\ FOR = 1 - NPV$

and we present the FDR as false positives per 100 positive tests and FOR as false negatives per 100 negative tests for prevalences ranging from 0 to 2%.



4.1 Figure 1. Flow diagram of the diagnostic algorithm
https://doi.org/10.1371/journal.pone.0180555.g001

Ethics approval and consent to participate

The protocol for this study was approved by the Ethical Review Committee of Ngaliema Clinic, Ministry of Public Health of the Democratic Republic of the Congo (approval number 184/ 2013). Written informed consent was obtained from all study participants with a positive screening test.

Results

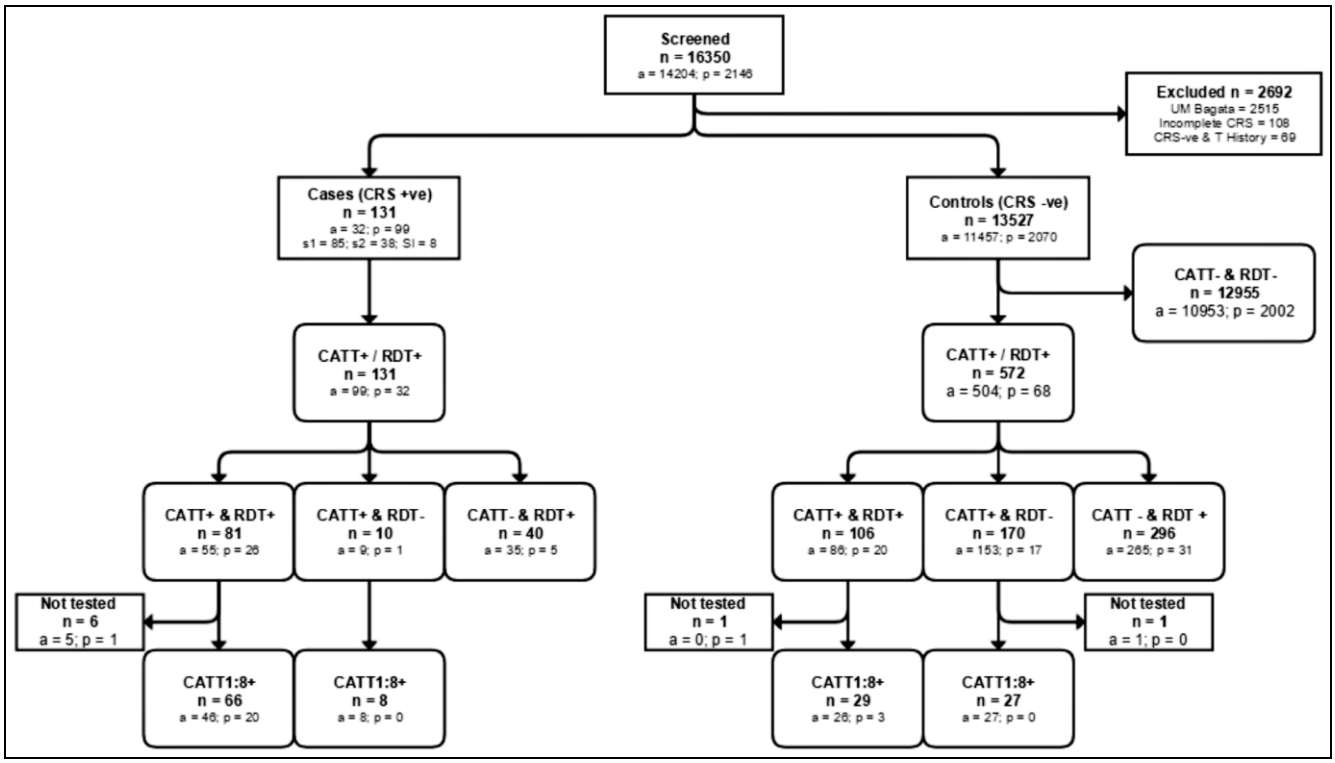
Enrolment of participants

Enrolment of participants was carried out over a period of 7 months, between 14 March 2013 and 7 October 2013 and the average duration of enrolment per site was 3 months. A total of 131 HAT cases were enrolled (99 through active screening and 32 through passive screening). Thirty-eight cases were in the second stage of disease, 85 were in stage 1. A lumbar puncture and CSF examination was not done on 8 confirmed cases, which for analysis, have been interpreted as stage 1 (Fig 2). Cases were diagnosed by gland puncture, mHCT and mAECT in almost equal proportions, while 4 cases were only positive by CSF examination.

The number of controls enrolled was 13,527, of whom 11,457 were through active screening and 2,070 through passive screening. Due to errors in recording of the screening results of sero-suspects that were not confirmed by parasitology by one mobile team, 2,515 potential controls that were enrolled by that mobile team were excluded (Fig 2). Another 108 could not be considered as controls because they were positive by CATT and/or the RDT, but either mHCT or mAECT were not performed, while 65 were excluded because they had a history of HAT.

Test sensitivity

The sensitivity of the RDT was 92.0% (95% CI = 86.1–95.5%), with band 2 (LiTat 1.5) recording a higher sensitivity than band 1 (LiTat 1.3) (Table 1). CATT on whole blood had a significantly lower sensitivity than the RDT (69.1%; 95% CI = 60.7–76.4%). CATT on plasma diluted 1:8 had a sensitivity of 59.0% (95% CI = 50.2–67.2%).



4.2 Figure 2. Flow diagram of participant enrolment.
Note that for illustrative purposes in this flow diagram, if either reader recorded a positive result then it is recorded as positive in this Figure.
https://doi.org/10.1371/journal.pone.0180555.g002

4.1. Table 1. Screening test sensitivity.

Test	Reader disagreement (%)	Cohen's Kappa (*p*-value)	Pos / N	Sensitivity (%) (95% CI)
RDT	0.8	0.948 (<0.001)	120.5 / 131	92.0 (86.1–95.5)
RDT band 1 (LiTat 1.3)	5.3	0.854 (<0.001)	99.5 / 131	76.0 (68.0–82.5)
RDT band 2 (LiTat 1.5)	3.8	0.861 (<0.001)	109.5 / 131	83.6 (76.3–89.0)
CATT whole blood	0.8	0.982 (<0.001)	90.5 / 131	69.1 (60.7–76.4)
CATT 1:8*	0	1 (<0.001)	74 / 125.5	59.0 (50.2–67.2)

Sensitivity of individual screening tests and agreement between readers as implemented in the field.
* 5 cases that were positive by CATT on whole blood by both readers, and 1 that was positive by reader 1, were not tested using CATT dilutions and were excluded from the section on agreement. For the sensitivity calculations, the denominator is 125.5, because one case had discordant results by CATT on whole blood for the two readers, and CATT dilution was not done on the case. As this case was negative by CATT whole blood by reader 2, it was a complete result for reader 2, but not for reader 1 as dilutions were not performed, and so we counted it as 0.5 of a case in the denominator.

https://doi.org/10.1371/journal.pone.0180555.t001

There was significant agreement between readers on all tests (Table 1), although for the RDT, the degree of disagreement between readers was higher on the individual bands than for the test as a whole. The RDT followed by CRS had the highest sensitivity in both mobile teams and fixed facilities, and among stage 1 and stage 2 HAT patients (Fig 3). The performance of the index test in different sites was variable (S1 File) and if the site that performed worst with CATT (Bagata mobile team) is removed from the analysis, then the sensitivity of CATT improves to 77.8% (95% CI = 67.6– 85.5%), but remains significantly less sensitive than the RDT (Chi-square p = 0.006).

Test specificity

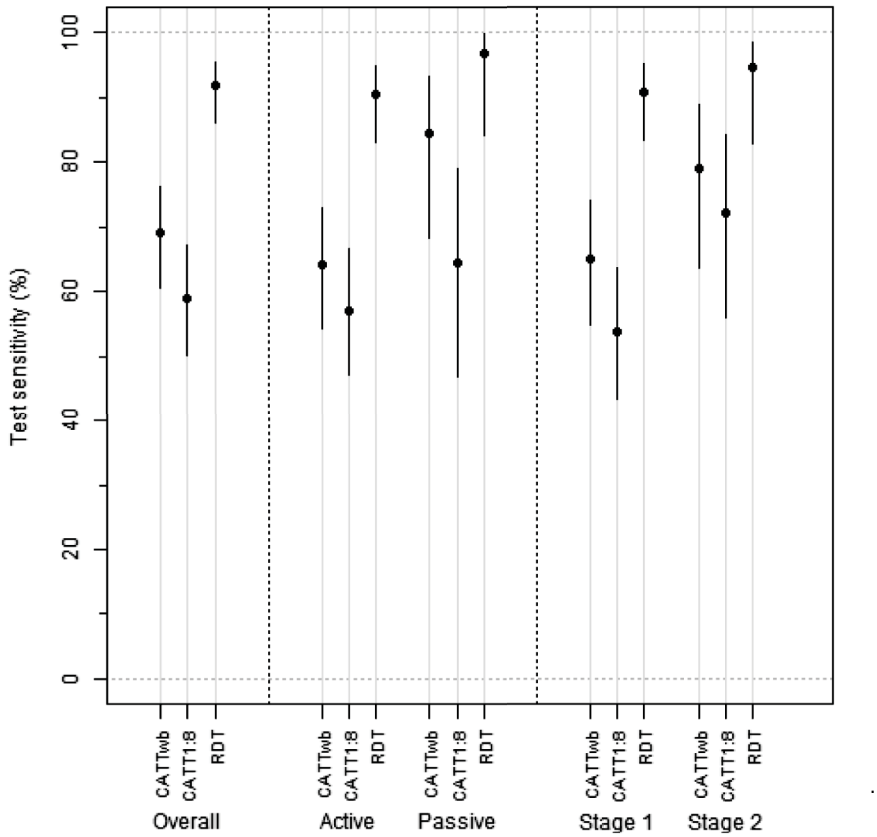
The specificity of all tests was greater than 97% and the inter-reader agreement was very good for all the tests (Table 2). The highest specificity was observed with CATT at 1:8 dilution, which was significantly greater than the specificity using either CATT on whole blood or the RDT (Table 2) and this did not vary between active and passive screening (Fig 4). There was relatively little variation in the performance of the index test in different sites in terms of specificity (S1 File).

Field application

In the field, at a 1% prevalence, 75.6% of RDT positives would be false positives, compared to 40.3% of positives found by CATT and CATT dilutions. The corollary of this is that for every 10,000 negative screening results (corresponding to approximately 40 active screening days), 8.3 would be false negatives (missed cases) by RDT, compared to 31.8 false negatives by CATT alone (Fig 5).

Agreement between tests

Approximately 50% of the HAT cases were positive by CATT and by both bands on the RDT (Table 3). This proportion was higher among stage 2 cases, 60–63% of whom were positive by CATT and both bands on the RDT, compared to 44–46% of stage 1 cases, although this difference was not statistically significant (Chi square $p > 0.1$). When the average of both readers was considered, 72.3% of the stage 2 patients were positive by both bands on the RDT, compared to 66.7% among stage 1 patients (Table 3).



4.3 Figure 3. Sensitivity of the tests.
Points represent the estimates and lines the 95% CIs. Active and passive refer to active and passive screening
https://doi.org/10.1371/journal.pone.0180555.g003

RDT band intensity

The following observations were made on the qualitative assessment of the intensity of RDT bands:

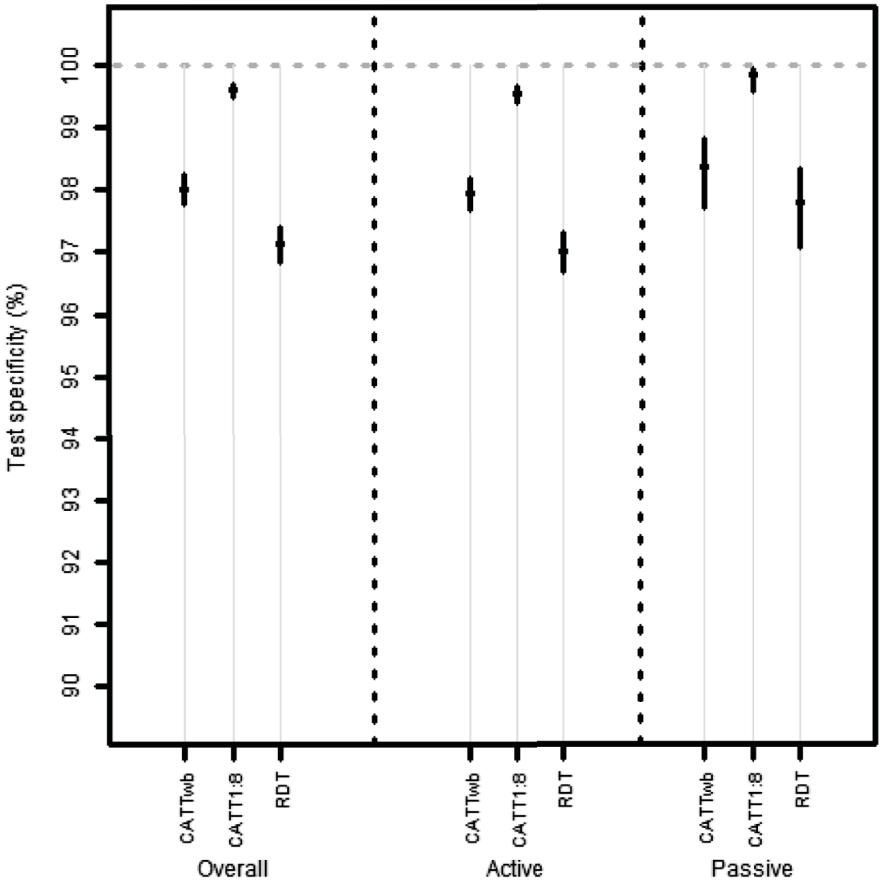
The intensity of RDT bands was stronger for HAT cases than for false positive suspects. The mean score on band 1 (LiTat 1.3) was 1.54 for cases, and 1.27 for false positive suspects, which was significantly lower (Wilcoxon rank sum test p = 0.003).

4.2 Table 2. Screening test specificity

Test	Reader disagreement (%)	Cohen's Kappa (p-value)	Neg / N	Specificity (%) (95% CI)
RDT	0.2	0.964 (<0.001)	13138.5 / 13527	97.1 (96.8–97.4)
RDT band 1 (LiTat 1.3)	0.3	0.934 (<0.001)	13256.5 / 13527	98.0 (97.8–98.2)
RDT band 2 (LiTat 1.5)	0.3	0.925 (<0.001)	13233.5 / 13527	97.8 (97.6–98.1)
CATT whole blood	0.1	0.970 (<0.001)	13259 / 13527	98.0 (97.8–98.2)
CATT 1:8*	0.01	0.990 (<0.001)	13470 / 13525	99.6 (99.5–99.7)

Specificity of individual screening tests and agreement between readers.
* Includes CATT whole blood negatives; 2 suspects who were positive by CATT on whole blood and were not subsequently tested by CATT dilutions were excluded.

https://doi.org/10.1371/journal.pone.0180555.t002

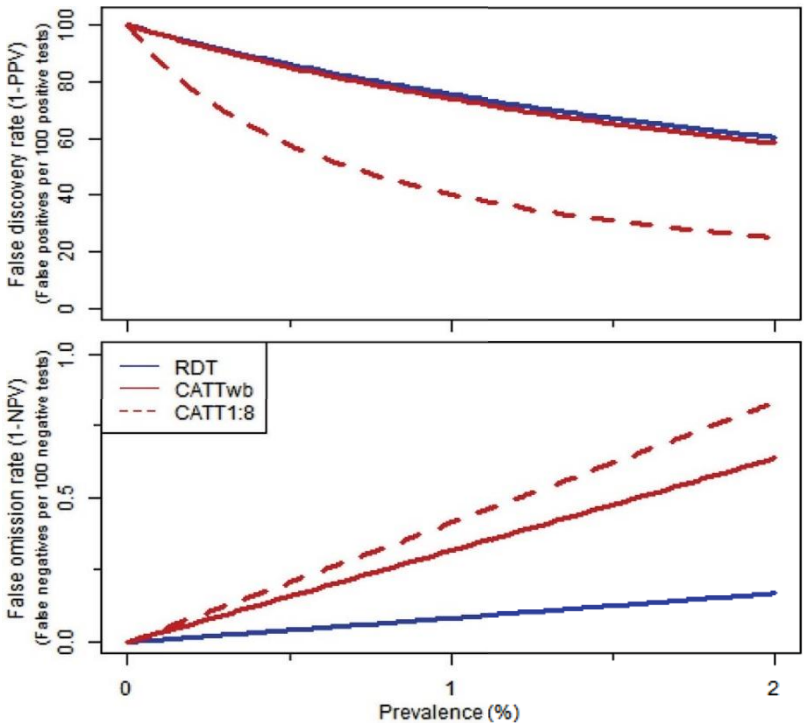


4.4 Figure 4. Specificity of the tests.
Points represent the estimates and lines the 95% CIs. Note the y-axis range is 90–100%. Active and passive refer to active and passive screening
https://doi.org/10.1371/journal.pone.0180555.g004

The mean score on band 2 (LiTat 1.5) was 1.68 for cases and 1.31 for false positive suspects, which was also statistically significant (Wilcoxon rank sum test $p < 0.001$).

- When band intensity scores of 1 were considered as negative, the sensitivity of the RDT decreased to 77.5% (95% CI = 69.6–83.8%) and the specificity increased to 97.9% (95% CI = 97.7–98.2%) and there was no significant difference with CATT for either sensitivity (Chi sq $p = 0.16$) or specificity (Chi sq $p = 1$).
- There was good agreement between readers on cases and false positive suspects. Both readers gave the same score for band 1 (LiTat 1.3) in 80.2% of cases, and for band 2 (LiTat 1.5) in 90.8% of cases. Among the false positive suspects, the agreement was slightly lower, at 75.9% and 77.6% respectively.

- The intensity score of both bands was the same among 54.2% of the cases for reader 1 and 49.6% of the cases for reader 2, compared to 24.6% and 29.9% for false positive suspects.
- Among the 402 identified as false positive suspects by the RDT, the largest proportion (16.2% and 17.1%) had an intensity score of 2 on band 2 (LiTat 1.5) and 0 on band 1 (LiTat 1.3) by the two readers. Among cases, 31.3% and 29.8% had an intensity score of 2 on each band for each reader.



4.5 Figure 5. False discovery and false omission rates.
FDR and FOR of each test followed by CRS over a range of prevalences, which were calculated based on the sensitivity and specificity of the algorithms that were found in this study
https://doi.org/10.1371/journal.pone.0180555.g005

Discussion

This study has demonstrated that the SD BIOLINE® HAT RDT had a higher sensitivity in both active and passive screening, with a difference of 23% between it and the second best screening test—CATT (Table 1 and Fig 3). However, this is at the expense of a slightly lower specificity of the RDT, which would result in some additional workload in confirmatory testing.

4.3 Table 3. Combination of positive screening tests

	Reader 1			Reader 2*		
	All (%)	**Stage 1 (%)**	**Stage 2 (%)**	**All (%)**	**Stage 1 (%)**	**Stage 2 (%)**
CATT & RDTB1 & RDTB2	67 (51.1)	43 (46.2)	24 (63.2)	63 (48.8)	40 (44.0)	23 (60.5)
CATT & RDTB1	4 (3.1)	2 (2.2)	2 (5.3)	5 (3.9)	3 (3.3)	2 (5.3)
CATT & RDTB2	10 (7.6)	8 (8.6)	2 (5.2)	12 (9.3)	9 (9.9)	3 (7.9)
RDTB1 & RDTB2	25 (19.1)	21 (22.6)	4 (10.5)	24 (18.6)	20 (22.0)	4 (10.5)
CATT	10 (7.6)	8 (8.6)	2 (5.3)	10 (7.8)	8 (8.8)	2 (5.3)
RDTB1	6 (4.6)	5 (5.4)	1 (2.6)	5 (3.9)	3 (3.3)	2 (5.3)
RDTB2	9 (6.9)	6 (6.5)	3 (7.9)	9 (7.0)	7 (7.7)	2 (5.3)
All negative	0	0	0	1 (0.8)	1 (1.1)	0
Total	**131**	**93**	**38**	**129**	**91**	**38**

Combinations of positive screening tests for the 131 HAT cases that were identified during this study. RDTB1 = RDT band 1; RDTB2 = RDT band 2.

* Two participants from reader 2 were excluded as the results of the qualitative assessment of band intensity were incomplete

https://doi.org/10.1371/journal.pone.0180555.t003

The simplicity and stability of the HAT RDT has created a great opportunity to improve screening coverage of the population at risk, as it can be deployed to any health facility in endemic areas. There were no issues regarding reproducibility–the agreement between readers was very good for both CATT and the RDT. However, there was a case that would have been missed if there was just one reader for each test, which highlights the importance of training and diligence of staff who are reading the tests.

The genome of *T. b. gambiense* codes for a large number of VSG antigens that are expressed differentially during the course of infection [26] and it has been reported that the VSGs LiTat 1.3 and LiTat 1.5 are predominantly expressed by *T.b. gambiense* [27]. The difference in sensitivity that we report here between the RDT and CATT could be due in part to the inclusion of the LiTat 1.5 antigen, and as such it could be assumed that a patient infected with trypanosomes that had expressed only the VSG LiTat 1.5 antigens might be missed by CATT and only detected using the RDT. Interestingly, there were more cases detected by band 2 of the RDT (VSG LiTat 1.5) than by band 1 (VSG LiTat 1.3) and the band 2 antigen was responsible for identifying 19 cases that would have been missed if only the band 1 antigen had been used in the RDT. Similar observations were made in a clinical trial on the prototype of the same RDT [21]. An explanation for this could be that widespread and continuous use of CATT as the main screening tool for several decades could have resulted in a strong selection pressure against parasites expressing LiTat 1.3 antigens. Previous studies have reported that some sub-populations of *T.b. gambiense* did not harbour the LiTat 1.3 gene [28], which would prevent detection using CATT. This in turn might explain the relatively low sensitivity of the CATT test that

was observed in this study, which would not have been identified without using another screening test based on different antigens and different presentation of the antigens to identify cases. Conducting a large study incorporating two screening tests has identified a number of cases that could have been missed in previous studies using CATT alone. It is therefore conceivable that including a third antigen in the RDT could further increase its sensitivity, but may also decrease specificity.

Most HAT cases were positive in both bands of the RDT. This suggests that at some point during infection, HAT cases had waves of parasitaemia with variant antigenic types (VATs) of parasites expressing each of LiTat 1.3 and LiTat 1.5 VSGs, and that the corresponding humoral response was maintained. This may also explain why stage 2 cases were more frequently positive by both RDT bands than stage 1 cases, as they were more likely to be infected for long enough to be exposed to multiple waves of parasitaemia (Table 3).

Another possible explanation for the observed difference in sensitivity between CATT and the RDT could be the difference in the format and chemistry of these tests. While the CATT test is based on the agglutination of freeze-dried fixed and stained trypanosomes with host antibodies, the RDT relies on the formation of a complex made of a nitrocellulose-bound antigen, host antibodies and a gold conjugated antigen. The antigens in the RDT are separated from the trypanosome, exposing other epitopes that in the fixed parasites used in the CATT test would remain hidden, such epitopes would bind other antibodies present in the patient, also contributing to better sensitivity. In addition, the composition of the dilution buffer that is used with the RDT is not publicly known, and it could be that it is different from that used in the CATT test, which could influence antigen-antibody binding.

During this study, CATT was used according to the manufacturer's instructions and staff performing the test ensured that both positive and negative controls reacted according to instructions and no failure to follow usage or storage protocols were observed. However, it remains possible that the antigen could have deteriorated within the limits of the positive control, resulting in decreased sensitivity, or the possibility of a weak agglutination that was not easily detected by the technicians. If there was deterioration, then this might explain some of the differences in CATT sensitivity between what was observed here and what was observed in a clinical study to evaluate the prototype RDT [21].

To estimate the true sensitivity of the RDT and CATT, it is necessary to identify all cases in the study population. Therefore, a weakness in this study is that it assumes that all cases would test positive with either CATT or RDT, but there could have been people infected with VATs that had not expressed any of the antigens in both tests. However, due to logistical challenges of screening such large numbers of people in a prospective study, it was not possible to perform parasitological confirmation on all subjects screened. As a consequence, subjects were only tested by parasitology if they were positive by RDT or CATT. If we were to assume that both CATT and RDT were independent, with sensitivities of 69.1% and 92.0%, then using both tests in parallel

and taking a positive on either test as a serological suspect would give a sensitivity of 97.5%, and as such if the tests were independent, then screening with both tests would result in missing 2.5% of cases. However, as the sensitivities of the two tests are not independent–due to the sharing of an antigen, the true combined sensitivity may be lower than 97.5% and more than 2.5% of cases missed. These missed cases and subsequent over-estimation of the sensitivity of the test is an unavoidable limitation of this study design. Consequently, the results of this analysis can be regarded as the conditional field sensitivity of the tests. Whilst the use of parasitological methods and other reference tests such as immune trypanolysis (TL) would be desirable, these techniques do not have 100% sensitivity, and would therefore still miss some cases [8,29].

In all HAT studies and screening programs, the specificity of the parasitological techniques is assumed to be 100%, but false positives have been reported in the past [30,31]. In this study, all HAT cases that were positive by parasitology were verified by a supervisor, thus minimising the risk of having false positives by parasitology, but there does remain a small risk of over-diagnosis. In terms of sensitivity, there is no reason to assume that false positives by parasitology would alter the comparisons between index tests. Whilst it would be desirable to perform TL as a reference test [8,29], it was not possible to collect samples for TL under this study design, but future studies should make efforts to incorporate TL.

The sensitivity and specificity of both CATT and RDT was higher in passive screening at fixed health facilities than in active screening, albeit not statistically significant. A possible explanation could be that there was a greater proportion of cases in stage 2 that were diagnosed at healthcare facilities. Due to their longer duration of infection, stage 2 cases would be more likely to have mounted an immune response to the VSGs used in these tests, resulting in higher sensitivity. The difference might also be due to sub-optimal blinding in healthcare facilities. Laboratory technicians in healthcare facilities are more likely to be aware of the clinical status of patients, mainly because patients are normally only screened for HAT at healthcare facilities if they have clinical signs, whereas at mobile teams, clinical signs are not considered prior to screening. This difference could have introduced a bias when interpreting the screening test results.

CATT dilutions had considerably greater specificity but lower sensitivity than CATT on whole blood and RDT. When these sensitivities are translated into case detection at the population level, the lower specificity of the RDT or CATT on whole blood during active screening leads to a larger number of suspects having to be taken through parasitological confirmation, which could be expensive and logistically challenging to screening teams. However, the corollary of this is that the improved sensitivity of the RDT followed by parasitological confirmation means that at 1% prevalence, there are around four times fewer false negative cases (missed cases) among those that test negative by the RDT than by the next most sensitive test (CATT). A balance would need to be struck in terms of positive and negative predictive values, which should be evaluated in a cost-benefit analysis for various prevalence values. From a practical perspective, a mobile team

usually screens between 200 and 300 people in one day. In a setting where the prevalence of HAT would be around 0.8%, an RDT with a specificity of 97% would detect a mean of 2 cases and 8 false positives per day. Although such a number is not large for a mobile team, the extra burden placed on the team and costs associated with testing the false positive suspects should be established.

This study was limited by the relatively small number of cases that were identified. Whilst the regions of the DRC that were selected for the study are among the most endemic, the prevalence of HAT in many parts of Africa has been falling, as was reflected in the prevalence of less than 1% among those that were enrolled in this study. A larger number of cases would have enabled us to get a more precise estimate of the sensitivity of the tests. With declining prevalence and corresponding decrease in positive predictive values, it could soon be necessary to develop diagnostics with even higher accuracy, to minimise the number of cases that are missed and achieve elimination, whilst minimising the workload and cost.

Conclusions

This study has demonstrated that the SD BIOLINE® HAT RDT has superior sensitivity when screening for *gambiense* HAT in both active and passive screening settings in the DRC. However, it still misses at least 8% of the cases, meaning that there remains scope for developing other screening tests with better performance. Other test combinations that were tested here offer better specificity, which would require further investigations in cost-effectiveness analysis to determine the optimal combination, especially for accelerated and sustained elimination of the disease. The RDT would also benefit from further testing in other settings. Serological tests with improved accuracy, if they can be developed, could then be used as true diagnostic tests, without the need for confirmation, and might also be used for the identification of asymptomatic carriers of HAT [32]. In the event that drugs that are safer and easier to use become available [33–35], a "test and treat" strategy would be feasible [36], thus accelerating elimination of the disease.

Supporting information

S1 File. Additional analysis. Additional analysis not included in the manuscript file. (PDF)

S2 File. Study protocol. The study protocol that was followed in the field. (PDF)

S3 File. Study data.

(XLSX)

S1 Table. STARD checklist. STARD checklist for the reporting of studies of diagnostic accuracy.

(PDF)

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4. Cost-effectiveness of using a rapid diagnostic test to screen for human African trypanosomiasis in the Democratic Republic of the Congo

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Abstract

New rapid diagnostic tests (RDTs) for screening human African trypanosomiasis (HAT) have been introduced as alternatives to the card agglutination test for trypanosomiasis (CATT). One brand of RDT, the SD BIOLINE HAT RDT has been shown to have lower specificity but higher sensitivity than CATT, so to make a rational choice between screening strategies, a cost-effectiveness analysis is a key element. In this paper we estimate the relative cost-effectiveness of CATT and the RDT when implemented in the Democratic Republic of the Congo (DRC). Data on the epidemiological parameters and costs were collected as part of a larger study. These data were used to model three different diagnostic algorithms in mobile teams and fixed health facilities, and the relative cost-effectiveness was measured as the average cost per case diagnosed. In both fixed facilities and mobile teams, screening of participants using the SD BIOLINE HAT RDT followed by parasitological confirmation had a lower cost-effectiveness ratio than in algorithms using CATT. Algorithms using the RDT were cheaper by 112.54 (33.2%) and 88.54 (32.92%) US dollars per case diagnosed in mobile teams and fixed health facilities respectively, when compared with algorithms using CATT. Sensitivity analysis demonstrated that these conclusions were robust to a number of assumptions, and that the results can be scaled to smaller or larger facilities, and a range of prevalences. The RDT was the most cost-effective screening test in all realistic scenarios and detected more cases than CATT. Thus, on this basis, the SD BIOLINE HAT RDT could be considered as the most cost-effective option for use in routine screening for HAT in the DRC.

Introduction

Human African trypanosomiasis (HAT) or sleeping sickness is caused by two subspecies of the protozoan parasite *Trypanosoma brucei*. This tsetse fly-transmitted disease is endemic in 36 sub-Saharan African countries. In recent years, the number of new cases of the chronic form of HAT caused by *T.b. gambiense* that were reported to the World Health Organisation (WHO) has decreased from 25,841 in 2000 to 2,131 cases in 2016. However, the actual number of cases is estimated to be much higher, as many patients remain undiagnosed or unreported [1]. The WHO roadmap on neglected tropical diseases (NTDs) of 2012 that was endorsed by the London Declaration of 2012 targets the elimination of HAT as a public health problem by 2020 [2].

A core component of HAT elimination strategies is the screening of large numbers of individuals who are at risk of infection, to identify and treat cases and break the transmission cycle [3–5]. This is because the clinical signs of HAT are non-specific, and prevalence of the disease in most regions is relatively low [6]. Furthermore, due to the relative toxicity of the drugs used and the onerous nature of the treatment, it is important to correctly identify infected individuals before they are given treatment.

Screening for HAT has traditionally been carried out using the card agglutination test for trypanosomiasis (CATT) [7]. Recently, two rapid diagnostic tests (RDT) have been commercialised and are being introduced in several endemic countries [8–10]. The RDT has advantages of being simple, easy to use, not requiring electricity, is instrument-free, and has a higher sensitivity than CATT when performed on whole blood ($CATT_{WB}$) but the RDT has lower specificity than $CATT_{WB}$, meaning that more false positives are identified during screening [11]. Some testing algorithms include performing CATT on serial dilutions of plasma, which improves specificity, but results in some cases being missed [7,12]. Furthermore, the current format of packaging makes it bulky to transport. Individuals who are positive by $CATT_{WB}$ or RDT (and referred to as screening suspects) must undergo further tests to confirm or rule out disease using a combination of parasitological tests. These consist of methods to identify parasites in various body fluids by microscopy, including blood, lymph node aspirates and the cerebrospinal fluid (CSF) [13]. After confirmation of disease, the CSF of HAT cases is examined by microscopy in a process known as staging, in order to determine the treatment to be used. In the DRC, routine parasitological tests comprise examination of lymph node aspirate (LN), micro-haematocrit centrifugation technique (mHCT) and the miniature anion exchange centrifugation technique (mAECT). In the DRC, active screening is carried out by mobile teams that visit communities in areas of high incidence. Although the majority (around 74%) of the local population typically presents for screening [6,14,15], this varies greatly between populations [6] and some high risk individuals may be missed [5]. Upon confirmation and staging, cases are referred to the nearest health facility that offers treatment. Individuals that are suffering from an illness may present to the local health facility, and if HAT is clinically

suspected, they are screened at that facility or are referred to the nearest facility that can screen for HAT. As prevalence declines and active screening programs are reduced, passive screening is becoming an increasingly important method of case detection in the DRC [7,8].

In view of the reported advantages and disadvantages of using either RDTs or CATT, it is vital to establish the relative cost-effectiveness of implementing different algorithms in different settings [16].

Materials and methods

A study was carried out in the DRC in 2013 to evaluate the performance of three algorithms for diagnosis of HAT during active screening by mobile teams and passive screening at fixed health centres [11]. Two algorithms used either the SD BIOLINE HAT RDT or $CATT_{WB}$ for screening, followed by a combination of routine microscopy tests. In the third algorithm, $CATT_{WB}$ was used for screening, and positives tested by CATT on plasma diluted 1:8. During the study, the tests were performed in parallel and the performance of algorithms subsequently evaluated. These testing algorithms were performed by four mobile teams and four hospitals and health facilities, located in three provinces in the DRC. From 16,480 people screened, 131 HAT cases were confirmed, and 13,526 controls identified [11].

We developed a model to calculate the number of cases diagnosed and the total costs of running three different HAT diagnostic algorithms in health facilities and mobile teams over one calendar year. The model was populated using epidemiological parameters and costs, and these were further tested using a number of sensitivity analyses.

In the model framework, the costs are estimated from the societal perspective, which includes all costs that are absorbed in the DRC, and excludes subsidies that are applied externally. The model we have developed evaluates the costs of screening, diagnosis and staging. The model does not include costs of treatment but for illustrative purposes, we evaluate the cost per DALY averted as if the diagnosed cases were treated, given the published treatment efficacy.

Model structure

Three testing algorithms that are currently in use in the DRC are modelled in this analysis, as summarised below. The parasitological testing algorithm is that in routine use in DRC (LN-mHCT-mAECT):

1. Screening with $CATT_{WB}$, confirmation by a combination of parasitological techniques and staging.
2. Screening with $CATT_{WB}$, further screening of positives by $CATT_{WB}$ using CATT on diluted plasma, Confirmatory testing of patients positive at a 1:8 dilution by a combination of parasitological techniques and staging.
3. Screening with RDT, confirmation by a combination of parasitological techniques and staging.

Estimation of the cost-effectiveness requires test and disease parameters as well as costs at different levels. The costs included comprise those of materials for performing the screening tests and the diagnostic tests, the capital costs for equipment, fixed costs such as staff wages, recurrent costs (annual and daily) and costs incurred by the individuals that presented for screening (travel costs and income foregone).

Thus, for each algorithm we considered:

1. The number of people that presented for testing, the cost of administering the screening test (materials and staff time) and the cost incurred by the individual to attend the test.
2. Based on disease prevalence and screening test accuracy parameters, the patients were grouped into four categories following screening:

a. *True positives = Number screened x prevalence x test sensitivity*

b. *False negatives = Number screened x prevalence x (1 - test sensitivity)*

c. *False positives = Number screened x (1- prevalence) x (1- test specificity)*

d. *True negatives = Number screened x (*1 – *prevalence) x test specificity*

3. False positives and true positives from screening were tested for the presence of parasites using the LN-mHCT-mAECT microscopy algorithm. True positives were tested sequentially with microscopy methods until parasites were demonstrated, or if parasites were not demonstrated, then the patient was a false negative (in addition to the screening false negatives). False positives were tested with the complete microscopy algorithm in order to confirm the absence of parasites. We accounted for the costs of the microscopy tests conducted (materials and staff time).

Assumptions

A number of rules and assumptions underpinning this model are detailed below:

1. Mobile teams are dedicated solely to diagnosis of HAT, while fixed facilities are involved in managing multiple diseases and medical conditions. Therefore, for mobile teams we consider the total cost for staffing and maintaining a mobile team for one year, whereas for fixed health facilities we consider the proportion of staffing resources that are used to test and confirm each HAT suspect and case.
2. The prevalence of HAT observed by mobile teams is an estimate of the "true prevalence", meaning that we assume it to be a representative sample of the local population at risk. This differs from the prevalence observed by fixed health facilities, where patients are screened based on a prior probability of infection. In fixed health facilities patients are selected either because they present due to symptoms or based on clinical suspicion established through consultation with a doctor or nurse. Therefore, we refer to detection at fixed health facilities as a case detection rate.
3. Diagnostic tests used for parasitological confirmation in these algorithms are independent. Data from a recent study of confirmatory tests in the DRC showed that the system sensitivity of LN-mHCT-mAECT was

77.9%, but if the tests were independent, the sensitivity would have been 90.0% [17], the sensitivity may also be boosted by CSF examination, when this is performed on the basis of clear clinical signs.

4. The combination of microscopy tests for confirming cases has 100% specificity. As the tests are all based on visualization of parasites, this is a reasonable and widely accepted assumption [14,18,19]. However, there is the potential for occasional operator error [20].

5. The RDT performed on whole blood and the CATT on serial dilutions are independent tests.

6. All the CATT reagents are used by mobile teams. The CATT is packaged such that one vial of CATT reagent is used to perform 50 tests. Once opened the reagent must be stored at 4–8°C and re-tested each day on positive and negative control samples and can get spoiled. In the case of mobile teams, we assume that at the end of each day, the remaining CATT reagent is stored and retains its potency until the following day. Therefore, we assume that 100% of available CATT tests are used by mobile teams.

7. All cost calculations are in US dollars (USD), and where necessary, converted using the 2013 exchange rates (S1 File).

8. Costs are considered from the perspective of the DRC, and only costs incurred within the DRC are included. The purchase (pre-shipment) cost of the SD BIOLINE HAT RDT to the DRC is 0.5 USD after a subsidy of 0.25 USD that is borne externally. In the study we consider the purchase cost of 0.5USD and this subsidy on RDTs is examined in sensitivity analysis.

9. Due to the packaging of the RDT, its transportation presents a challenge. Accordingly, we assumed that additional transportation equipment was required for the algorithm involving RDTs in mobile teams and include costs accordingly.

Diagnostic test and epidemiological parameters

Wherever possible, parameters for sensitivity and specificity of diagnostic tests were taken from a clinical trial of the SD BIOLINE HAT RDT [11] and compared to other published estimates (Table 1). We placed an emphasis on alternative estimates that are from the DRC whenever these were available, particularly on data from the national HAT control program in the DRC (PNLTHA). Any parameter estimated from the SD BIOLINE HAT RDT clinical trial that differed markedly from previously published estimates, or was subject to variability, was tested in sensitivity analysis.

In this model, mobile teams screen for 220 days per year, by working for 20 days followed by 10 days of rest each month for 11 months in a year, with one full month off duty. Fixed facilities operate for 250 days per year, consisting of 52 working weeks and 10 public holidays. To calculate the payment per hour from an annual salary, we assume that staff work 2,000 hours per year. This is based on an average of 40 hours per week for 52 weeks minus 10 public holidays.

Costs

Details of the derivation and calculation of the costs used in these analyses are given as supplementary information (S1 File). The costs are broken down as follows:

1. Capital costs: These are the costs of purchasing the equipment necessary to run the mobile team or the HAT screening at a fixed unit. Capital costs are calculated using the straight line depreciation method over a 5-year useful life for mobile teams and 20 years for fixed health facilities. Total annual capital costs for a mobile team were 12,001 and 12,781 USD for mobile teams implementing CATT and RDTs respectively, and 551 and 496 USD for a fixed health facility for CATT and RDTs respectively.
2. Annual recurrent costs: For mobile teams, these include the costs of staffing the unit for one year (staffing costs for fixed health facilities are calculated on a per test basis), the costs of training, insurance and maintenance. Total costs for mobile teams are 30,307 USD / year and 435 USD / year for fixed health facilities.
3. Daily running costs: These include the costs of consumables such as fuel, water and stationery. For mobile teams, they also include the allowances paid to staff. For mobile teams, the total costs are 97 USD per screening day (21,340 USD / year) and for fixed health facilities 3.50 USD per screening day (875 USD / year or 0.35 USD per patient screened, based on screening 10 patients per day).
4. Staffing costs for fixed health facilities: The cost for a laboratory technician is 0.78 USD / hour and 0.63 USD / hour for a nurse based on national salaries. We assume that the consultation at a fixed health facility is with a nurse, although in practice this could occasionally be with a doctor.
5. The costs of materials (including shipping) for the diagnostic tests are: CATTWB is 0.70 USD in mobile teams and 0.76 USD in fixed facilities (the cost at the fixed facility includes a small loss due to repeating control tests), RDT 0.60 USD (0.85 excluding a 0.25 USD subsidy, but including 0.1 USD shipping cost), CATT dilutions 3.02 USD; LN 0.38 USD; mHCT 1.54 USD; mAECT 7.20 USD; materials for CSF examination are 2.00 USD.
6. The time required to perform each test was (in minutes): clinical consultation 20; CATTWB 10; RDT 17; CATT dilutions 15; aspiration of lymph nodes and examination of aspirate 15; mHCT 18; mAECT 30; examination of CSF 30.
7. We included costs incurred by individuals presenting for screening, including travel costs and costs of missed work. These were 0.32 USD for mobile teams and 1.97 USD for fixed health facilities

5.1 Table 1. Diagnostic tests and epidemiological parameters.

Parameter	Estimate (95% CIs)*	Alternative observations (source)	Comments (sources)
HAT prevalence	0.82% (0.68 - 0.98)	0 - 2% (PNLTHA)	Based on case detection rates at active screening
HAT incidence rate among individuals reporting for passive screening	1.76% (1.28 - 2.42)	0 - 5% (PNLTHA)	
Proportion of HAT cases in stage 2 Health facilities Mobile teams	 50.0% (33.6 - 66.4) 22.2% (15.2 - 31.4)		
Number presenting for screening to the mobile teams	250 per day	120 - 350 (PNLTHA)	
Number presenting for screening at health facilities	10 per day	1 - 30 (PNLTHA)	Smaller facilities will test fewer individuals
Sensitivity RDT	92.0% (86.1 - 95.5)		
Specificity RDT	97.1% (96.8 - 97.3)		
Sensitivity $CATT_{WB}$	69.1% (60.7 -76.4)	87% - 98% [6]	91.2% used for comparison [19]
Specificity $CATT_{WB}$	98.0% (97.8 - 98.3)	83.5 - 99.3% [7]	97.4% used for comparison [19]
Sensitivity CATT on 1:8 diluted plasma	59.2% (50.4 - 67.4)		77.6% used for comparison [19]
Specificity CATT on 1:8 diluted plasma	99.6% (99.5 - 99.7)		99.1% used for comparison [19]
Proportion with palpable lymph nodes HAT cases HAT suspects	 39.7% (31.7 - 48.3) 11.7% (9.3 - 14.6)		Sensitivity among those with palpable lymph nodes. The overall sensitivity is 28.2%.
Sensitivity LN	71.2% (57.7 - 81.7)		Sensitivity among those with palpable lymph nodes. The overall sensitivity is 28.2%.
Sensitivity mHCT	55.2% (45.3 - 64.8)	56.5% [14] 52.1% [17]	
Sensitivity mAECT	78.3% (66.4 - 86.9)	75.3% [14] 68.1% [17]	
Efficacy stage 1 treatment (Pentamidine)#	99.0%		Source [14]
Efficacy stage 2treatment (NECT)#	96.0%		Source [21]
Iatrogenic mortality stage 1 treatment (Pentamidine)#	0.1%		Source [14]
Iatrogenic mortality stage 2 treatment (NECT)#	1.0%		Source [21]

Data were sourced from the SD BIOLINE HAT RDT clinical trial [11], unless otherwise stated.
These are included for the estimation of DALYs averted.

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Implementation and analysis

The model is implemented in the R statistical environment [22]. From the model, the following values are calculated:

1. The total cost of implementing the surveillance activities for one year.
2. The number of people screened in one year.
3. The number of people that were infected with HAT among the number screened.
4. The number of false positives from screening that then undergo confirmatory testing by microscopy.

5. The number of false negatives after screening and confirmatory testing (missed cases), this comprises both false negatives from screening that did not proceed for confirmatory testing as well as false negatives following confirmatory testing.
6. The number of HAT cases that were diagnosed.
7. For the estimation of DALYs the number of HAT cases that are subsequently cured assuming that all cases present for treatment and considering the published treatment efficacy. This is calculated as the number of true positives that were successfully treated (number of confirmed HAT cases in each stage of HAT that were successfully treated given the treatment efficacy, and the iatrogenic mortality due to treatment for stage 1 and stage 2 of HAT). The corresponding number of disability adjusted life years (DALYs) averted is calculated, considering 28.6 and 25.1 DALYs averted for a true positive treated in stage 1 and stage 2 respectively [23] (S1 File).

From this we calculate the average cost-effectiveness ratio (ACER) as the total cost per HAT case diagnosed, and the incremental cost-effectiveness ratio (ICER) as the difference between each algorithm and the algorithm that was most cost-effective by ACER. We also calculate the cost per disability adjusted life year (DALY) averted, considering 28.6 and 25.1 DALYs averted for a true positive treated in stage 1 and stage 2 respectively [23]. The DALYS are discounted and age weighted, calculated using life expectancies from the DRC (S1 File).

Sensitivity analysis

A number of sensitivity analyses are carried out to test the robustness of the model to the parameters outlined above. Specifically, we test:

1. A range of sensitivities of the $CATT_{WB}$ and RDT from 60–100% and specificities from 90–100%.
2. Revised $CATT_{WB}$ sensitivity of 91.2% and specificity of 97.4% based on a review [19]. Accordingly, the sensitivity of CATT at 1:8 dilution following a positive $CATT_{WB}$ test is 85.1% and specificity 63.6% following a positive $CATT_{WB}$ test [19].
3. Revised RDT and CATT sensitivity and specificity based on a recent paper comparing three screening tests [24]. The paper found comparable specificities, but considerably lower sensitivities estimated separately for passive and active screening. The sensitivities of the RDT were 49.2 and 70% in active and passive screening respectively. For CATT they were 51.8 and 74.6% respectively. Specificities of the RDT were 99.4 and 96.7% in active and passive screening and for CATT 99.5% and 97.6% respectively. The study also included a second generation of RDT with a sensitivity in active screening of 54.8% and 90.1% in passive screening. Corresponding specificities are 99.1% and 93.7%. This second generation RDT has the same purchase cost of 0.5USD.
4. The cost of the RDT without the subsidy of 0.25 USD.
5. Prevalence of HAT among the population that presents for screening ranging from 0.1% - 2%.

6. The number presenting for screening at fixed health facilities, from 1 patient per day (to represent smaller facilities) to 30 patients per day to represent the largest hospitals in highly endemic areas.

7. Variations in the numbers presenting daily for screening to a mobile team from 120 to 350.

8. An additional cost to the patient for presenting for confirmation from fixed health facilities, to reflect algorithms that are used in Uganda and Kongo Central in DRC [8,10] where patients may be referred for confirmation. Supplementary sums ranging from 1 - 20 USD are considered.

Of the 7 analyses listed above, only the first three will influence the dominance of one algorithm over another. The remainder change the overall costs of all algorithms equally.

CHEERS checklist

The analyses is consistent with the Consolidated Health Economic Evaluation Reporting Standards (CHEERS) guidelines [25,26]. The completed checklist is S1 Table.

Ethics statement

The protocol for the clinical trial on the RDT was approved by the Ethical Review Committee of Ngaliema Clinic, Ministry of Public Health of the Democratic Republic of the Congo (approval number 184/2013). Written informed consent was obtained from each participant before enrolment in the study. In the case of children, informed consent was obtained from a parent or guardian.

Results

The optimal algorithm measured by the ACER, by the cost per DALY averted, and by the number of deaths averted for both mobile teams and fixed health facilities is the RDT followed by a combination of microscopy tests (Table 2). In terms of the ICER, in mobile teams the algorithm using the RDT strongly dominated (it was cheaper and diagnosed more cases) the algorithm with $CATT_{WB}$. The algorithm using the RDT weakly dominated (it cost more but diagnosed more cases) the algorithm with CATT dilutions. In fixed health facilities, the algorithm using the RDT weakly dominated both alternatives. Implementation of any of the algorithms in a fixed facility always had a better ACER than they did when implemented in a mobile team.

In fixed health facilities the majority of the costs are those for screening and costs incurred by patients (Table 3). In mobile teams the majority of the costs are annual costs (including staff wages) and screening (Table 3). Participant costs accounted for 48.60% and 13.51% of costs at fixed health facilities and mobile teams respectively.

Sensitivity analysis

When the sensitivity and specificity of the $CATT_{WB}$ is adjusted to be similar to that reported in previous studies [19] the ACER of all algorithms involving CATT closes in on that of the RDT, but the algorithm comprising the RDT remains most cost-effective in both mobile teams and fixed health facilities (Table 4).

5.2 Table 2. Summary of cost-effectiveness analysis results.

Algorithm	Screening false positives (%)	Cases diagnosed (%)	HAT cases cured (%)	Total cost	USD / DALY averted	ACER	ICER
Mobile teams; 55,000 screened; 449.5 HAT +ve							
$CATT_{WB}$	1080.8 (2.0)	288.9 (64.3)	283.3 (63.0)	130351	16.8	451.2	-0.876
$CATT_{WB}$ + $CATT_{1:8}$	220.6 (0.4)	251.5 (56.0)	246.6 (54.9)	126811	18.7	504.2	25.95
RDT	**1566.7 (2.8)**	**384.7 (85.6)**	**377.2 (83.9)**	**130267**	**12.6**	**338.6**	**-**
Fixed health facilities; 2,500 screened; 44.0 HAT +ve							
$CATT_{WB}$	48.7 (1.9)	28.3 (64.3)	27.4 (62.3)	10115	13.4	357.6	1.98
$CATT_{WB}$ + $CATT_{1:8}$	9.9 (0.4)	24.6 (56.0)	23.9 (54.3)	9986	15.2	405.5	11.31
RDT	**70.5 (2.8)**	**37.7 (85.6)**	**36.5 (83.0)**	**10133**	**10.1**	**269.1**	**-**

The cost-effectiveness of 3 algorithms modelled in the 2 screening strategies over one year of screening. The two optimal algorithms are shown in bold. $CATT_{1:8}$: CATT is performed on serially diluted plasma samples, using the 1:8 dilution as cut-off.

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When varying the sensitivities of the two basic tests is considered at health facilities the $CATT_{WB}$ becomes more cost-effective than the RDT if the sensitivity of the RDT is below 68.9%, or if the sensitivity of $CATT_{WB}$ is above 92.2% (Fig 1). The corresponding values at mobile teams are 68.8% and 92.3%

Considering screening test specificity, the CATT becomes more cost-effective at health facilities when the specificity of the RDT is below 96.0%, or the specificity of CATT is above 98.5% (Fig 2). The corresponding specificities at mobile teams are 96.2% and 98.3% (Fig 2).

Using the CATT and RDT results of [24] with a sensitivity of the RDT that is below that of CATT then the ACER of CATT in active screening is $568 and of RDT is $577. In passive screening the respective ACERs are $335 and $356. An algorithm including screening with the second generation RDT followed by confirmation and staging would have an ACER of $525 in active screening and $296 in passive screening.

When the 0.25 USD subsidy on the SD BIOLINE HAT RDT is not included, the ACER for the RDT followed by microscopy in mobile teams is 381.85 USD. It is 285.70 at fixed health facilities.

At all plausible HAT prevalences, the RDT remains more cost-effective than CATT, but converges at higher prevalences, when the CATT parameters from [19] are used (Fig 3).

5.3 Table 3. Cost breakdown.

	RDT + microscopy fixed health facilities			RDT + microscopy mobile team		
Description	**Cost**	**% total cost**	**Cost per case diagnosed**	**Cost**	**% total cost**	**Cost per case diagnosed**
Capital costs	496.10	4.90	13.58	12,781.20	9.81	33.89
Annual costs	435.00	4.29	11.91	30,307.00	23.27	80.36
Daily costs	875.00	8.63	23.96	21,340.00	16.38	56.58
Screening costs	2,577.50	25.44	70.58	33,000.00	25.33	87.50
Participant costs	4,925.00	48.60	134.86	17,600.00	13.51	46.67
Confirmation costs	824.75	8.14	22.58	15,238.52	11.70	40.40
	10,133		277.48	13,0267		345.39

Breakdown of the costs incurred to implement the most cost-effective algorithms by mobile teams and at fixed health facilities.

https://doi.org/10.1371/journal.pone.0204335.t003

5.4 Table 4. Summary of cost-effectiveness analysis results with revised parameters for $CATT_{WB}$.

Algorithm	**Screening false positives (%)**	**Cases diagnosed (%)**	**HAT cases cured (%)**	**Total cost**	**USD / DALY averted**	**ACER**	**ICER**
Mobile teams; 55,000 screened; 449.5 HAT +ve							
$CATT_{WB}$	1418.3 (2.6)	381.4 (84.8)	373.9 (83.2)	133671	13.0	350.5	-1037
$CATT_{WB}$ + $CATT_{1:8}$	516.3 (0.9)	332.0 (73.9)	325.5 (72.4)	131024	14.7	394.6	-14.38
RDT	**1566.7 (2.8)**	**384.7 (85.6)**	**377.2 (83.9)**	**130267**	**12.6**	**338.6**	-
Fixed health facilities; 2,500 screened; 44.0 HAT +ve							
$CATT_{WB}$	63.9 (2.6)	37.3 (84.8)	36.2 (82.3)	10297	10.4	275.8	-507.7
$CATT_{WB}$ + $CATT_{1:8}$	23.2 (0.9)	32.5 (73.9)	31.5 (71.6)	10224	11.8	314.5	-17.63
RDT	**70.5 (2.8)**	**37.7 (85.6)**	**36.5 (83.0)**	**10133**	**10.1**	**269.1**	-

The cost-effectiveness of 3 algorithms modelled in the 2 screening strategies over one year of screening with a $CATT_{WB}$ sensitivity of 91.2% and specificity 97.4%. The two optimal algorithms are shown in bold. $CATT_{1:8}$: CATT is performed on serially diluted plasma samples, using the 1:8 dilution as cut-off.

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The RDT remains optimal across a range of plausible numbers of people screened per day at both health facilities and mobile teams (Fig 4).

When additional costs incurred by the patient of being referred from health facilities for confirmatory diagnosis are factored in, the RDT remains more cost-effective. This remains until the cost to the patient exceeds 41.05USD at the basic prevalence and 40.19USD at a prevalence of 0.05% (Fig 5).

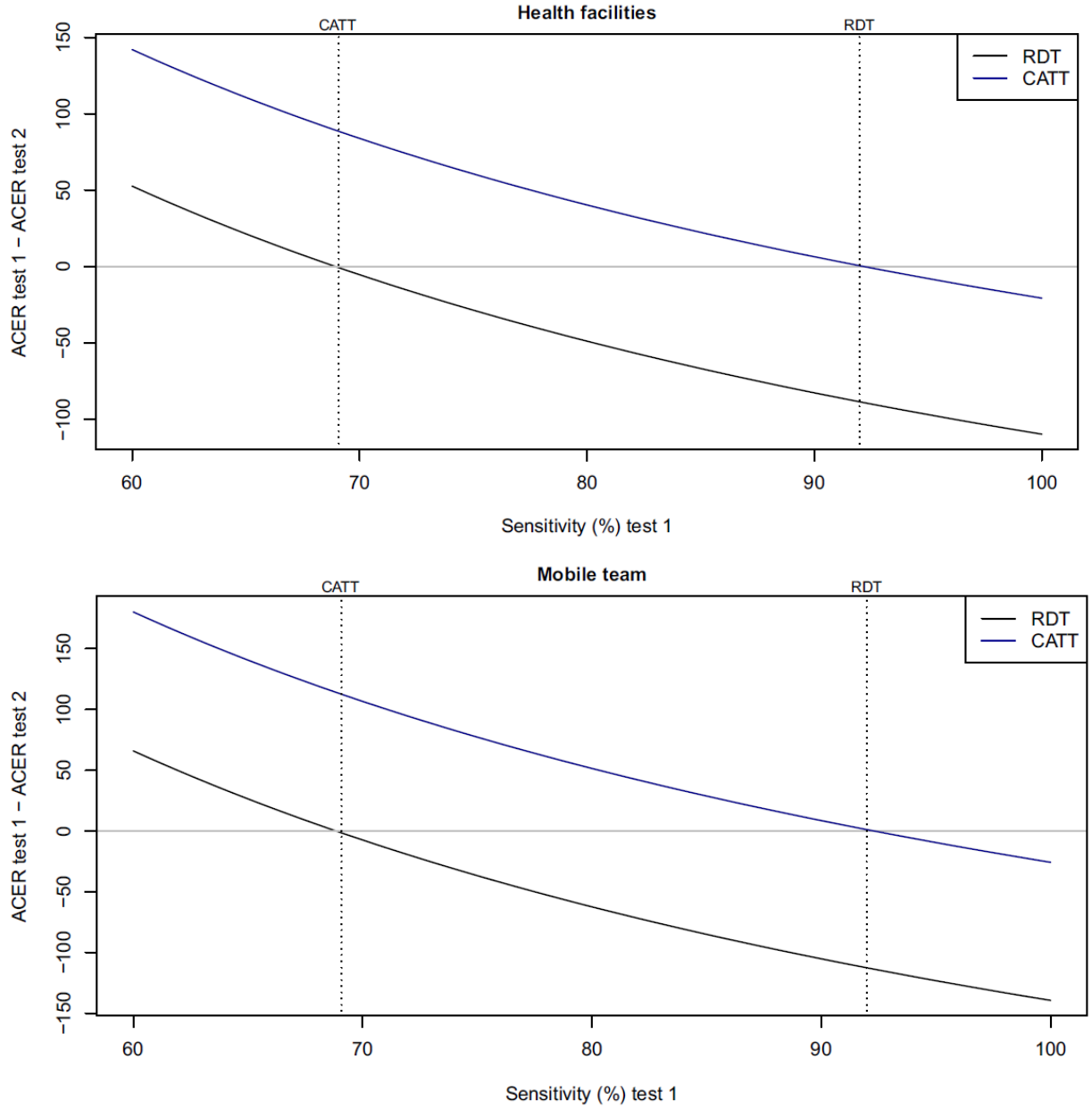
Discussion

This study demonstrates that the most cost-effective algorithm for either active or passive screening for HAT is one that includes the SD BIOLINE HAT RDT followed by microscopy. In addition to being more cost-effective, this algorithm is also the most effective in terms of the percentage of cases that are diagnosed and the DALYs that are averted. As well as being more cost-effective than CATT, the RDT has the added

advantages of greater practicality by being a single use format and not requiring electricity and a cold chain [27,28]. These findings are relevant to the DRC in terms of the costs and test performance parameters.

The findings presented here are generally robust to most sensitivity analyses. The sensitivity of CATT during the clinical trial on the SD BIOLINE HAT RDT was lower than many previous estimates [19,29] but the RDT is still the most cost-effective when the analysis are re-run with the alternative $CATT_{WB}$ parameter estimates. However, using the sensitivity and specificity estimates from a study of three tests [24] that had a sufficiently large sample size to give separate estimates for active and passive screening CATT had a marginally lower ACER than the SD BIOLINE HAT RDT in both active and passive screening. Based on this study, a second generation RDT was estimated to be more cost effective than both the first generation RDT and CATT in both active and passive screening.

When the subsidy of 0.25 USD for the SD BIOLINE HAT RDT is not considered, then the RDT is less cost-effective than $CATT_{WB}$ at the higher sensitivity, but the RDT remains more effective in terms of the percentage of cases diagnosed. In the future, it may be possible to further reduce the costs of manufacture and shipping by modifying test format and by packing many tests in a small volume, which would be particularly attractive for active screening. Whilst difficult to quantify, the additional transportation burden of the test in its current format was allowed here by including an additional 5,000USD to the purchase cost of a vehicle.



5.1 Figure 1. ACER at varying sensitivities.
We vary the sensitivity parameter of one of the tests (test 1 = the solid lines) and show the difference in ACER between test 1 and the reference test (test 2) whose sensitivity parameter is fixed at the values in Table 1. The RDT is compared to $CATT_{WB}$ and vice-versa. The broken lines show the sensitivity of the reference tests

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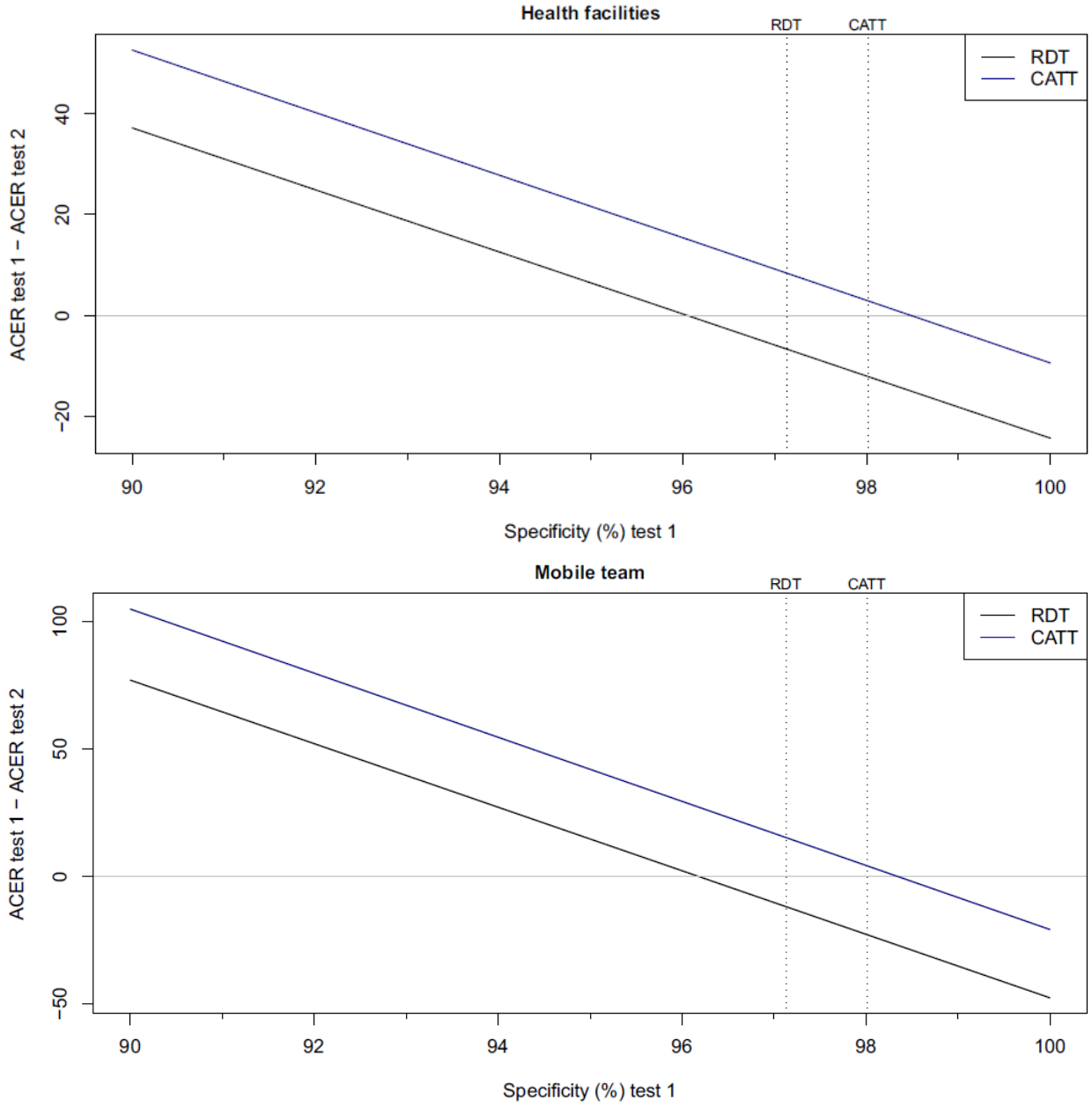
The lower specificity of the RDT relative to $CATT_{WB}$ increases the cost, due to higher costs of performing microscopy on the false positives identified after screening. This is principally because all screening suspects that are false positives must be tested with all microscopy methods including mAECT to confirm an absence of parasites. By using this more expensive test, the confirmation



5.2 Figure 2. ACER at varying specificities.

We vary the specificity parameter of one of the tests (test 1 = the solid lines) and show the difference in ACER between test 1 and the reference test (test 2) whose specificity parameter is fixed at the values in Table 1. The RDT is compared to CATTWB and vice-versa. The broken lines show the specificity of the reference tests

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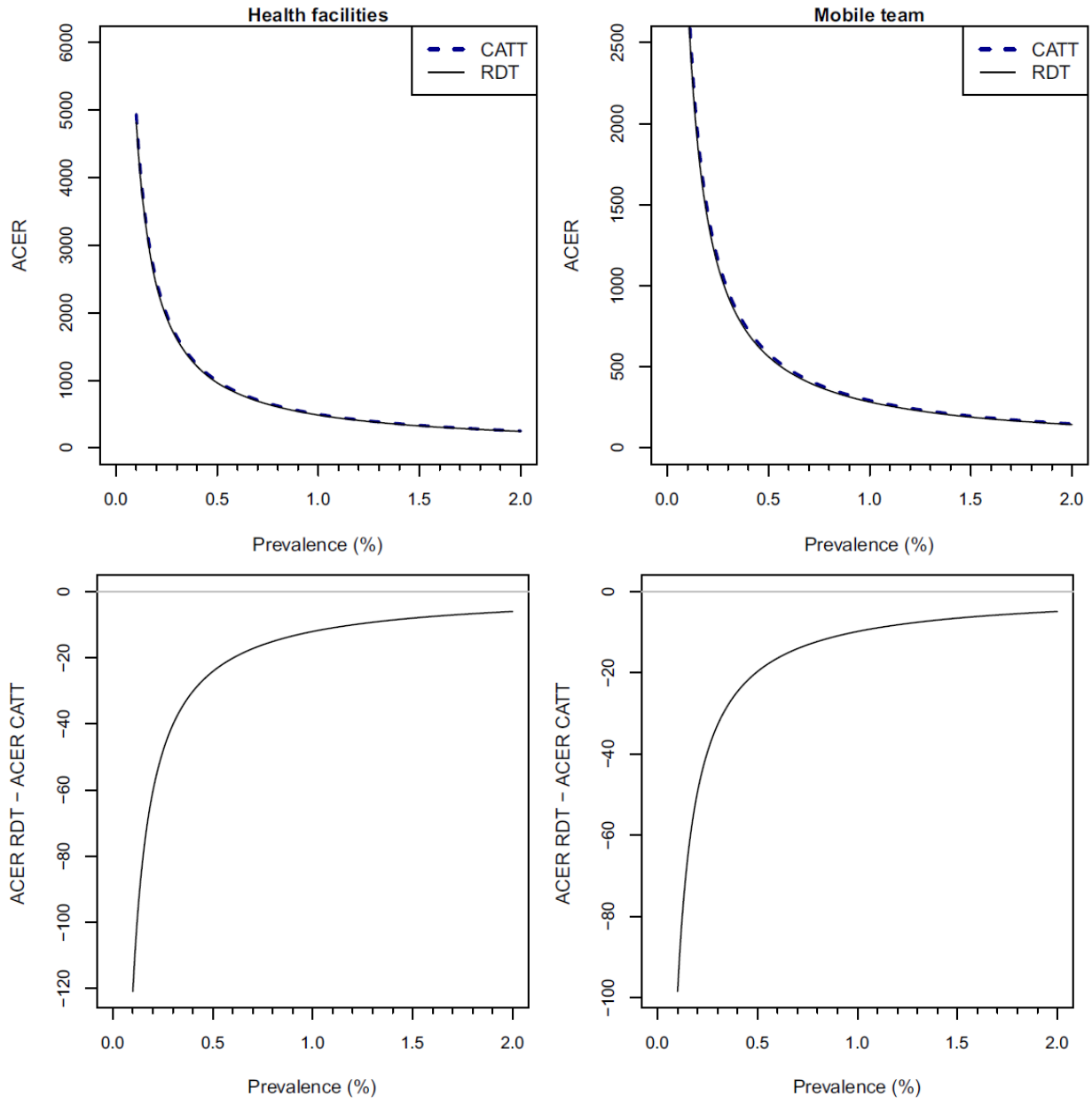
costs for the RDT algorithm were around 40% higher than those for $CATT_{WB}$, but the RDT remained cost-effective. Despite reducing the number of individuals.



5.3 Figure 3. ACERs at varying HAT prevalences.

We vary the prevalence parameter from 0.1–2% with the sensitivity and specificity of CATTWB set at 91.2% and 97.4%, The top two plots show the ACERs of CATTWB and the RDT in health facilities and mobile teams. The bottom two plots show the difference between the ACERs for the two tests at health facilities and at mobile teams.

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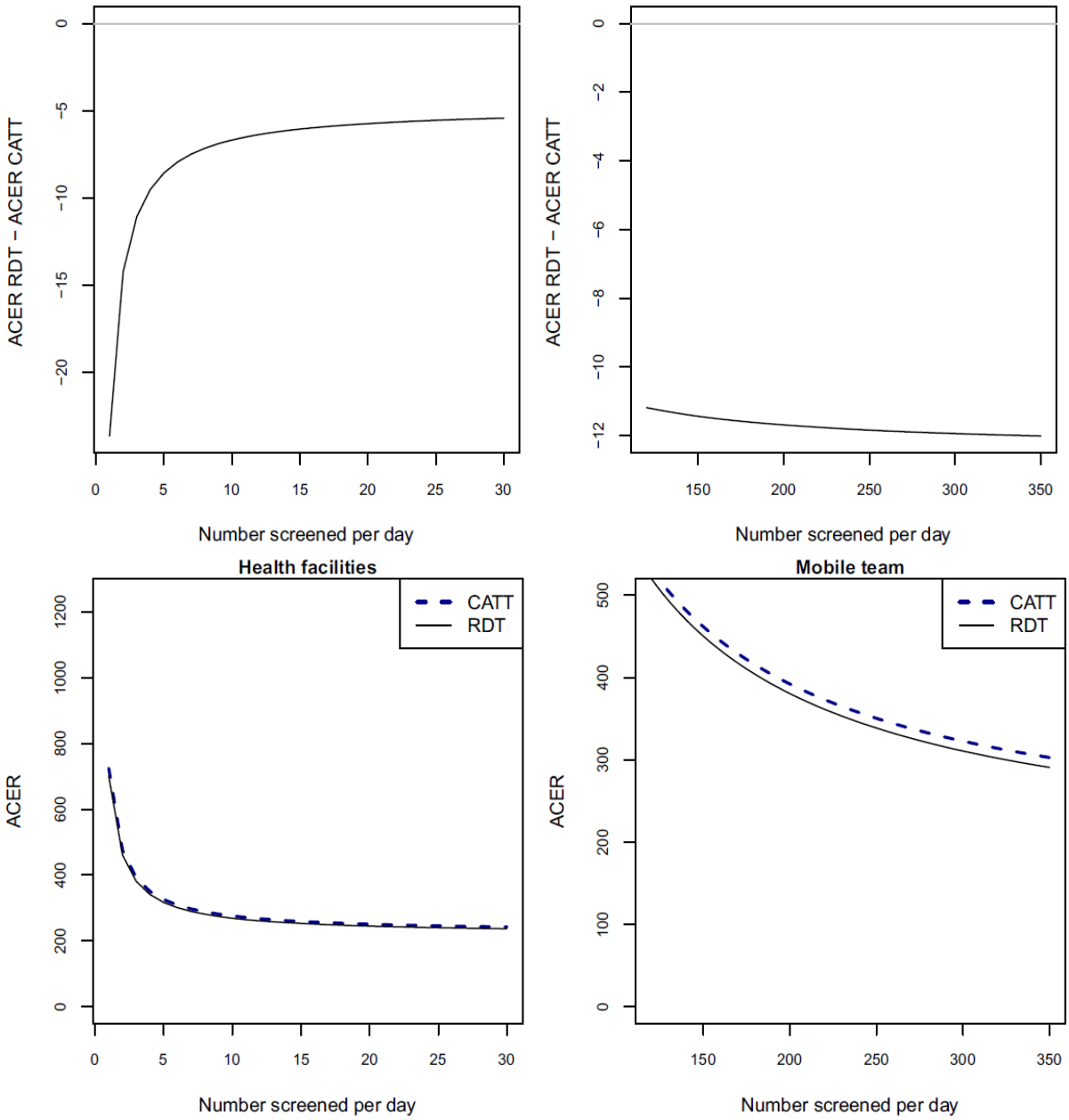
that proceed to the microscopy tests, CATT dilutions did not result in an improvement in the cost-effectiveness of any algorithm, due to the poor sensitivity of CATT on 1:8 diluted plasma.



5.4 Figure 4. ACERs at varying numbers screened.

We vary the numbers screened at health facilities from 1–30 and at mobile teams from 120–350 with the sensitivity and specificity of CATTWB set at 91.2% and 97.4%, The top two plots show the ACERs of CATTWB and the RDT in health facilities and mobile teams. The bottom two plots show the difference between the ACERs for the two tests at health facilities and at mobile teams.

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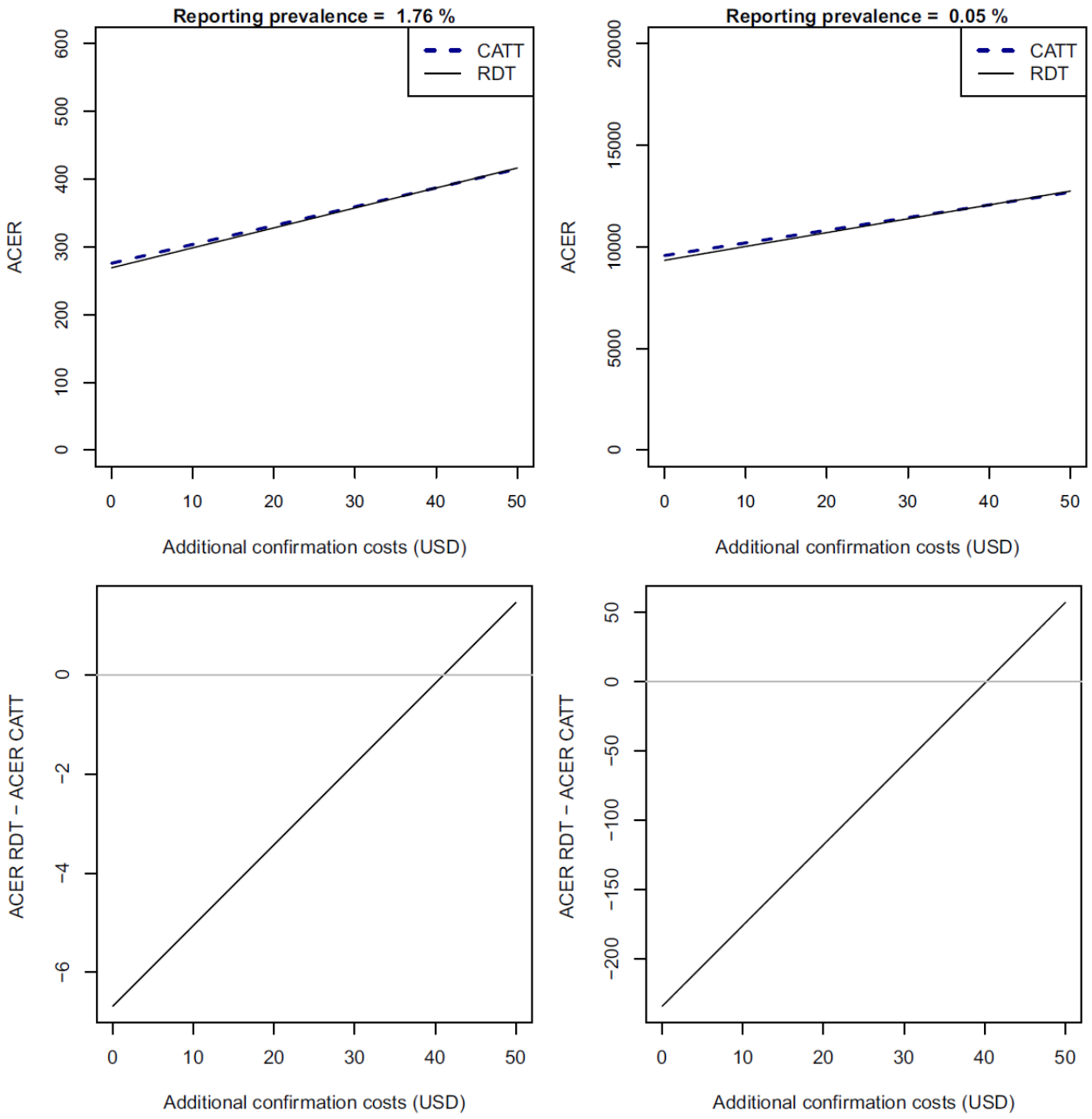
As HAT nears elimination, a number of foci are adopting a passive surveillance strategy in which HAT suspects are screened at a number of facilities that perform the HAT RDT. RDT positive patients (suspects) are referred to the nearest facility with the capacity to perform confirmatory diagnosis by microscopy [8,10].



5.5 Figure 5. ACER at different patient confirmation costs.
At different reporting prevalences at health facilities, the variation in ACER for a range of costs to a patient that is referred for confirmation with the sensitivity and specificity of CATTWB set to 91.2% and 97.4%. The top plots show the ACERs for the RDT and CATTWB for two different reporting prevalences and the bottom plots the difference between the ACERs.
https://doi.org/10.1371/journal.pone.0204335.g005

In these algorithms, the patient usually bears the cost of this referral, and therefore a less specific test such as the RDT may be less cost-effective so more false positives from screening would be referred. In these analyses, we demonstrate that this is not the case unless this cost exceeds 40USD (Fig 5).

This study has shown that active case finding by mobile teams is less cost-effective but active screening will continue to play a significant role in disease elimination. This is particularly the case in areas where there are a low number of health facilities and so the access of the population to screening is poor [30]. Conventional vehicle based active screening is also being augmented by new strategies such as targeted screening and motorcycle-based screenings that may improve cost effectiveness by reducing costs or the numbers of non-infected individuals that are screened [31]. Furthermore, as active surveillance does not rely on cases becoming ill and presenting at a health facility it can identify cases earlier during infection [7,15,32]. Consequently, active case finding identifies a larger proportion of cases in stage 1 when morbidity is lower and treatment easier and safer, many infections are sub-clinical, and the patient has been infectious for a shorter period. Nevertheless, as prevalence falls, numbers presenting for active screening are also likely to fall, requiring more innovative methods of identifying cases [3,31].

Conclusions

This study demonstrates that algorithms for screening for HAT that use the SD BIOLINE HAT RDT are the most cost-effective in both mobile teams and fixed health facilities, at the prices applicable in the DRC and sensitivities and specificities experienced there. The RDT is also the most effective in terms of case detection. Furthermore, we have provided evidence that such algorithms will remain the most cost-effective as HAT nears elimination, and the numbers presenting for screening declines.

Supporting information

S1 File. Cost and parameter derivation. This file gives details of the calculation of costs and parameters used in this study. (DOC)

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5. Prospective evaluation of a rapid diagnostic test for *Trypanosoma brucei gambiense* infection developed using recombinant antigens.

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Abstract

Background

Diagnosis and treatment are central elements of strategies to control *Trypanosoma brucei gambiense* human African trypanosomiasis (HAT). Serological screening is a key entry point in diagnostic algorithms. The Card Agglutination Test for Trypanosomiasis (CATT) has been the most widely used screening test for decades, despite a number of practical limitations that were partially addressed by the introduction of rapid diagnostic tests (RDTs). However, current RDTs are manufactured using native antigens, which are challenging to produce.

Methodology/Principal findings

The objective of this study was to evaluate the accuracy of a new RDT developed using recombinant antigens (SD BIOLINE HAT 2.0), in comparison with an RDT produced using native antigens (SD BIOLINE HAT) and CATT. A total of 57,632 individuals were screened in the Democratic Republic of the Congo, either passively at 10 health centres, or actively by 5 mobile teams, and 260 HAT cases were confirmed by parasitology. The highest sensitivity was achieved with the SD BIOLINE HAT 2.0 (71.2%), followed by CATT (62.5%) and the SD BIOLINE HAT (59.0%). The most specific test was CATT (99.2%), while the specificity of the SD BIOLINE HAT and SD BIOLINE HAT 2.0 were 98.9% and 98.1%, respectively. Sensitivity of the tests was lower than previously reported, as they identified cases from partially overlapping sub-populations. All three tests were significantly more sensitive in passive than in active screening. Combining two or three tests resulted in a markedly increased sensitivity: When the SD BIOLINE HAT was combined with the SD BIOLINE HAT 2.0, sensitivity reached 98.4% in passive and 83.0% in active screening.

Conclusions/Significance

The recombinant antigen-based RDT was more sensitive than, and as specific as, the SD BIOLINE HAT. It was as sensitive as, but slightly less specific than CATT. While the practicality and cost-effectiveness of algorithms including several screening tests would need to be investigated, using two or more tests appears to enhance sensitivity of diagnostic algorithms, although some decrease in specificity is observed as well.

Author summary

Sleeping sickness, or human African trypanosomiasis (HAT), is a neglected tropical disease that represents a risk to more than seventy million people in Sub-Saharan Africa. Most cases are caused by infection with *Trypanosoma brucei gambiense*. Diagnosis of HAT relies on the identification of suspected cases by serological methods, which include recently developed rapid diagnostic tests (RDTs). Current RDTs are produced using native antigens that are purified from live parasites in a laborious and dangerous process. The objective of this study was to evaluate the performance of a new RDT made using recombinant antigens, by screening people in fifteen endemic sites in the Democratic Republic of the Congo. The new RDT was found to be more sensitive than, and as specific as, the

reference RDT made using native antigens. It was also more sensitive than CATT, a serological test that has been widely used for decades. While one third of HAT cases were correctly diagnosed by all tests, the other cases were only identified by one or two of the tests. In order to enhance case detection and accelerate elimination of HAT, there may be a need to explore diagnostic strategies that combine two or more screening tests.

Introduction

Human African trypanosomiasis (HAT) is a vector-borne, neglected tropical disease, which puts 70 million people living in sub-Saharan African countries at risk [1]. The most common form of the disease is caused by infection with the protozoan parasite *Trypanosoma brucei gambiense* (g-HAT), which in 2015, accounted for more than 97% of all reported HAT cases [2]. Patients progress from an early disease stage that is characterized by the presence of trypanosomes in the blood and lymphatic system, to a late stage that is associated with the invasion of the central nervous system by parasites [3]. If left undiagnosed and untreated, the disease is generally fatal, although asymptomatic cases and others that progress spontaneously to apparently pathogen-free status have been reported [4].

Identification of serological suspects is the main entry point into diagnostic algorithms for g-HAT. The card agglutination test for trypanosomiasis (CATT/*T.b. gambiense*) has been the most commonly used screening test for g-HAT. It detects antibodies using a suspension of purified, fixed and stained bloodstream-form trypanosomes expressing LiTat 1.3 variant surface glycoprotein (VSG), a predominant variant antigen of *T.b. gambiense* [5]. While CATT has played a central role in the control of HAT, its large-scale implementation for passive screening in health facilities in remote locations has been limited due to operational challenges such as the need for an agitator, electricity and refrigeration. In some settings, the sensitivity and specificity of CATT have also been reported as being problematic [6].

In an effort to address the shortcomings of CATT, two rapid diagnostic tests (RDTs) that detect host antibodies have recently been developed, the HAT Sero-*K*-SeT manufactured by Coris BioConcept (Belgium), and the SD BIOLINE HAT, hereinafter referred to as "RDT1", produced by Alere/Standard Diagnostics (SD, South Korea), which include the same two antigens, VSG LiTat 1.3 and VSG LiTat 1.5. These RDTs were evaluated in retrospective studies, with very promising performance results [7,8]. Evaluation of a prototype of the RDT1 in a prospective study in three endemic countries, Angola, the Democratic Republic of the Congo (DRC) and the Central African Republic, showed that the sensitivity of the RDT was not different from the sensitivity of CATT, while its specificity was 1.3% lower [9]. A prospective study using the HAT Sero-*K*-SeT also reported excellent performance [10]. A comparison of both RDTs in an independent study using stored plasma samples collected in Guinea and Côte d'Ivoire concluded that there was no difference in diagnostic accuracy between the two tests [11]. The RDTs have now been introduced in multiple HAT endemic countries, where they are being used in HAT elimination programmes. However, production of the native antigens used in the manufacture of the RDTs remains a challenge, as it relies on a labor-intensive, costly and risky process that involves inoculating rats with human-infective trypanosomes. To address this challenge, and to improve standardization and quality of manufacturing, a new RDT that is produced exclusively using recombinant antigens, the SD HAT BIOLINE 2.0

("RDT2"), has been developed in a partnership facilitated by the Foundation for Innovative New Diagnostics (FIND).

The primary objective of this study was to evaluate the diagnostic accuracy of RDT2 in a multi-centric, prospective study in the DRC, and to demonstrate its non-inferiority to RDT1. As a secondary objective, the accuracy of RDT2 was compared to that of CATT.

Methods

Enrolment of participants

Study participants were enrolled from 6 June 2015 to 5 January 2016 in the Bandundu Province of the DRC by passive screening in ten health facilities, and by active screening using five mobile teams of the Programme National de Lutte contre la Trypanosomiase Humaine Africaine (PNLTHA) of the DRC (Table 1). In the health facilities, participants were enrolled among patients presenting themselves or referred from other health facilities after suspicion of HAT, and among relatives who accompanied patients. During active screening, anybody who presented to the mobile team was eligible for enrolment in the study. Study sites were visited by an external monitor prior to commencement of the study to verify that they were adequately prepared and personnel properly trained, and during the study to verify that the protocol was being adhered to. HAT cases were defined as subjects in whom trypanosomes were demonstrated by microscopy in either lymph node aspirate, blood or cerebrospinal fluid (CSF). All positive parasitology results were verified by the site supervisor. Cases were classified as early stage when no trypanosomes were observed in their CSF, and the CSF white cell count was lower than or equal to 5 cells/μL, while those with trypanosomes in the CSF and/or a cell count above 5 cells/μL were classified as late stage [12]. Controls were subjects living in the same areas as cases, with no known history of HAT infection, and who were either negative with all three screening tests, or who were positive with one or several screening tests, but in whom no parasites were detected in any body fluid. Clinical signs and symptoms were not considered exclusion criteria for controls.

6.1 Table 1. Study sites and the corresponding numbers of HAT cases and controls that were enrolled.

Site name	Site type	HAT cases	Controls
Nkara	Fixed facility (HS)	41	1,698
Masi-Manimba	Fixed facility (HGR)	31	1,521
Masamuna	Fixed facility (CS)	18	2,041
Kwamouth	Fixed facility (HGR)	8	784
Kitoy	Fixed facility (HS)	8	951
Bagata	Fixed facility (HGR)	5	420
Yasa Bonga	Fixed facility (HGR)	5	761
Bangumi	Fixed facility (CS)	3	321
Bandundu	Fixed facility (CDTC)	3	1,184
Bandundu	Fixed facility (HGR)	0	934
Kwamouth	Mobile team	45	7,326
Mushie	Mobile team	39	7,279
Bandundu	Mobile team	20	19,457
Idiofa	Mobile team	21	7,737
Mokala	Mobile team	13	3,855
All sites		*260*	*56,269*

HS : "Hôpital Secondaire"; HGR: "Hôpital Général de Référence"; CS: "Centre de Santé"; CDTC: "Centre de Dépistage, Traitement et Contrôle".

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Tests performed.

The RDT2 (SD, South Korea) is an immuno-chromatographic test for qualitative detection of antibodies of all isotypes (IgG, IgA and IgM). It includes a nitrocellulose membrane strip with two test regions (T1 and T2) that are pre-coated with two recombinant antigens. T1 is coated with Invariant Surface Glycoprotein 65 - 1 (ISG65) expressed in *Escherichia coli* [13] and T2 with the N-terminal domain of Variant Surface Glycoprotein LiTat 1.5 (VSG LiTat 1.5) produced using a Baculovirus expression system. A procedural control line (C) is also included. The test is stable for at least 24 months at 40°C, or 5 weeks at 55°C. The test is performed in the same way as RDT1, as described by Lumbala *et al*. [14]. In summary, a sample of 20 μl of whole blood is taken from a finger prick and transferred into a sample well using a disposable plastic capillary tube, and 4 drops (approximately 120

μl) of test diluent are then added. The sample flows along the membrane by capillarity, passing through the test regions T1 and T2. Results are read after 15 to 20 minutes by comparing the intensity of the test lines against a colour chart provided by the manufacturer. A result is considered positive when the control line C and either one or both T1 and T2 test lines are visible (regardless of their intensity), negative when only the C line is observed, and invalid if the C line is not observed. In active screening, all participants found positive with a HAT screening test were also tested for malaria using an RDT (SD BIOLINE Ag *P.f*), while in passive screening, all participants were tested with a malaria RDT (S3 Table). However, results of malaria RDTs were only recorded for subjects who were eligible for enrolment (see below). Those who tested positive for malaria were examined, and if necessary, treated in line with national guidelines.

Three screening tests (CATT, RDT1 and RDT2) were performed on finger-prick blood from each subject who presented to mobile teams, any subject who presented to a health facility with symptoms indicative of HAT, and accompanying individuals who consented to participate in the study. The results of screening tests were read by two independent laboratory technicians or nurses, and the results recorded separately. To avoid overburdening study teams and to keep the study design as simple as possible, CATT was only performed on whole blood, and not on diluted plasma. Similarly, the trypanolysis test was not performed during this study, as this would have required additional resources to collect and transport samples for analysis, which at that time could not be performed in DRC.

In both active and passive enrolment, any subject who was positive with at least one of the screening tests, or who showed symptoms highly suggestive of HAT, was eligible for immediate enrolment in the subsequent parasitological work-up. Written informed consent was sought from these subjects prior to enrolment. Any individual who declined to participate in the study was managed according to the standard procedures of the PNLTHA. Individuals who were negative to all three screening tests and who had no symptoms highly suggestive of HAT were not investigated further. Persons with palpable cervical lymph nodes had a lymph node aspirate taken and examined for motile parasites by bright field microscopy. A sample of 5 ml of venous blood was collected from each participant in a heparinized tube. Three hundred μL of blood was used to perform the capillary tube centrifugation (CTC) test (4 capillary tubes of approximately 65–70 μL) [15]. If the result of CTC was negative, 500 μL of whole blood was used to perform the mini anion exchange centrifugation technique (mAECT-wb) [16] and the remaining volume of blood (4.2 ml) was centrifuged to perform mAECT on buffy coat (mAECT-bc) as described by Camara et al. [17]. Since the mAECT-bc procedure had only been evaluated in one study in DRC, we took advantage of this study to collect some additional performance data to compare it with mAECT-wb, even though mAECT was only performed on a subset of cases. A lumbar puncture was performed on all HAT cases confirmed by any of the parasitological methods, as well as on other participants with clinical signs that were strongly suggestive of HAT, according to routine procedures. Parasitological examination of CSF was done

using the modified single centrifugation technique [16]. The technicians who performed the tests were employees of the PNLTHA, with experience in performing routine parasitological tests for detection of trypanosomes. Training of personnel of mobile teams and fixed health facilities included how to perform, read and interpret results of the RDTs, the study protocol and related SOPs, completion of CRFs and data management. Any positive or doubtful parasitology result was verified and confirmed by the site supervisor. Participants with any missing screening test or parasitology results were excluded from the study. All the HAT cases that were identified during the study were treated according to national guidelines.

Two levels of blinding were adopted. During the initial screening of participants using blood from a finger prick, three health workers were each responsible for performing one of the three screening tests. The health workers operated independently (but used blood from the same finger prick), without exchanging results (first level of blinding), and did not have access to any clinical information. A supervisor was responsible for collecting results of the tests and deciding whether or not to collect venous blood for parasitological tests.

Samples of venous blood were labelled with blinding codes by the supervisor (second level of blinding). The same codes were used to identify all samples collected from the participants (i.e. blood, lymph node aspirate, CSF) and constituted the anonymization process that was maintained throughout the entire study.

Data management and statistical analysis

Participant information and test results were recorded at study sites on paper case report forms, which were transferred to PNLTHA in Kinshasa for double data entry using a web-based clinical data management platform (VisionForm). Since two independent readings were available for each test and each sample, an approach based on bootstrapping resampling [18] was adopted: At each iteration, a random sequence of readings from the available data was generated (one reading per patient and per test) and used to calculate the performance metrics. This process was repeated (2,000 iterations per metric) to generate an empirical distribution of values for each metric, from which it was possible to derive values for the sample mean and 95% confidence intervals as bootstrapped percentiles.

Estimates of sensitivity and specificity were calculated for each screening test, on the overall data, and stratified by disease stage and by screening method (i.e. active and passive screening). The diagnostic performance of each antigen in the RDTs was also calculated. Sensitivity and specificity were defined as the percentage of HAT cases that were found positive and the percentage of controls that were found negative, respectively. Accuracy was assessed by calculating Youden's index [19]. To evaluate the agreement between readers, Cohen's Kappa factor was calculated. The statistical analysis was performed in the R statistical environment (version 3.2.3).

Sample size calculation

The sample size was calculated to demonstrate non-inferiority of the sensitivity and specificity of RDT2 in comparison to RDT1. Based on the sensitivity of RDT1 of 92.0% that was reported by Lumbala et al. [14], using

a non-inferiority margin of 8%, a confidence level of 5% and a power of 80%, the required number of HAT cases was calculated to be at least 143. Based on the same report, the expected specificity of RDT1 was 97.1%. Using a non-inferiority margin of 1%, a confidence level of 5% and a power of 90%, it was calculated that a minimum of 4,775 controls would be needed [20]. Based on the expected prevalence of HAT in the study area, the minimum number of subjects estimated to be screened in order to enroll 143 cases was 44,700.

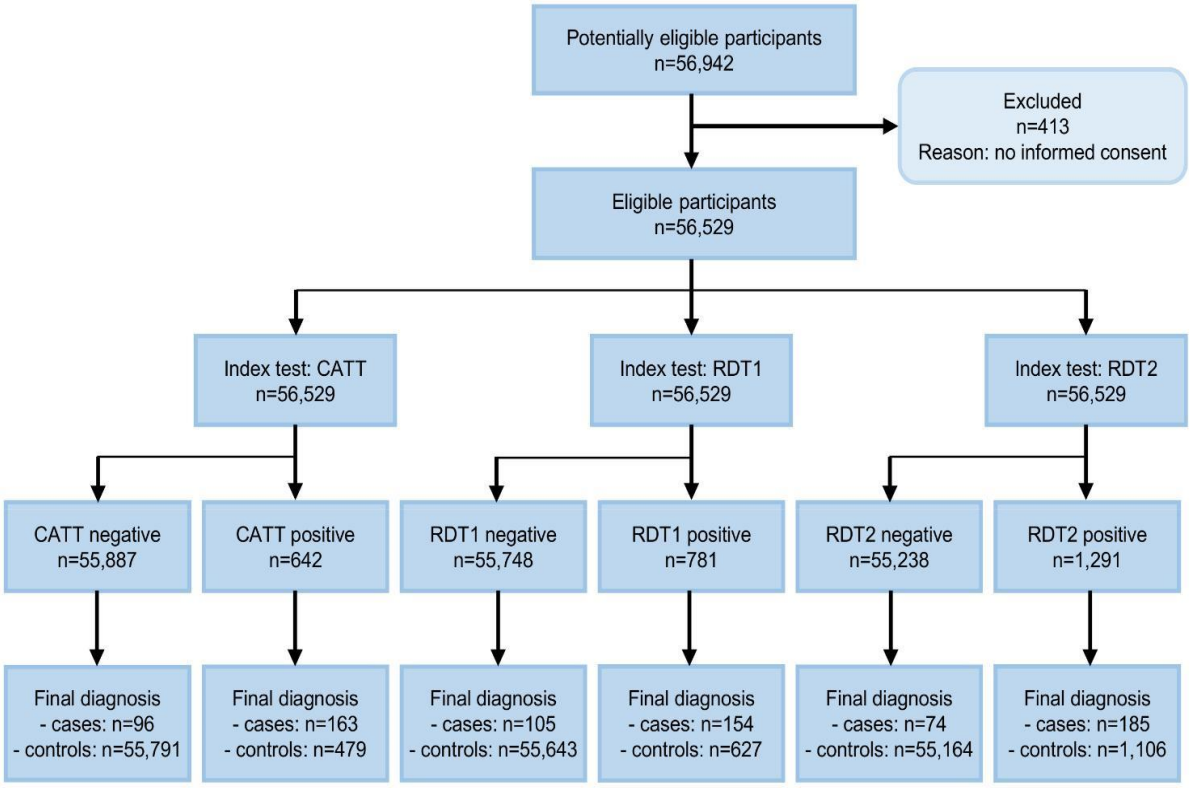
Ethical considerations

The study received ethical clearance from the School of Public Health of the University of Kinshasa (authorization number ESP/CE/012/2015). Participants provided written informed consent before being enrolled in the study. For children below 18 years, consent was provided by a parent or guardian. All individuals who presented at study sites during the period of enrolment and consented to being screened were eligible. Those who presented for screening but did not wish to participate in the study were screened according to the procedures of the PNLTHA. All participants' samples were blinded and further analyzed anonymously.

Results

A total of 260 HAT cases and 56,269 controls were enrolled after screening 56,942 people. 413 individuals could not be included in the study because they did not provide informed consent (Fig 1). A total of 138 (53%) cases and 45,654 controls were enrolled by active screening, while 122 (47%) cases and 10,615 controls were enrolled by passive screening. Among cases, the early stage to late stage ratio was 4.3 in active screening and 0.53 in passive screening. The HAT prevalence was 0.30% in active and 1.13% in passive screening. On average, 255 persons were tested per day by each mobile team.

The estimates of sensitivity, specificity and accuracy of the RDT2, RDT1 and CATT tests in active screening, passive screening, and active and passive screening combined are shown in Fig 2. When the results of active and passive screening were combined, the sensitivity of the three screening tests was unexpectedly low. While RDT2 detected 71.2% [CI: 65.7%; 76.6%] of the HAT cases, CATT detected only 62.5% [CI: 56.2%; 68.4%] and RDT1 only 59.0% [CI: 53.0%; 64.6%] of the cases. Sensitivity was particularly low in active screening, with only 54.8% [CI: 46.8%; 63.5%], 51.8% [CI: 43.1%; 59.9%] and 49.2% [CI: 40.9%; 57.6%] of cases being detected by the RDT2, CATT and RDT1, respectively. In passive screening, the three tests were more sensitive, with RDT2 achieving the highest sensitivity (90.1% [CI: 84.7%; 95.3%]), followed by CATT (74.6% [CI: 66.7%; 82.3%]) and RDT1 (70.0% [CI: 61.5%; 77.9%]).



6.1 Figure 1. STARD diagram describing the flow of participants through the study.
https://doi.org/10.1371/journal.pntd.0006386.g001

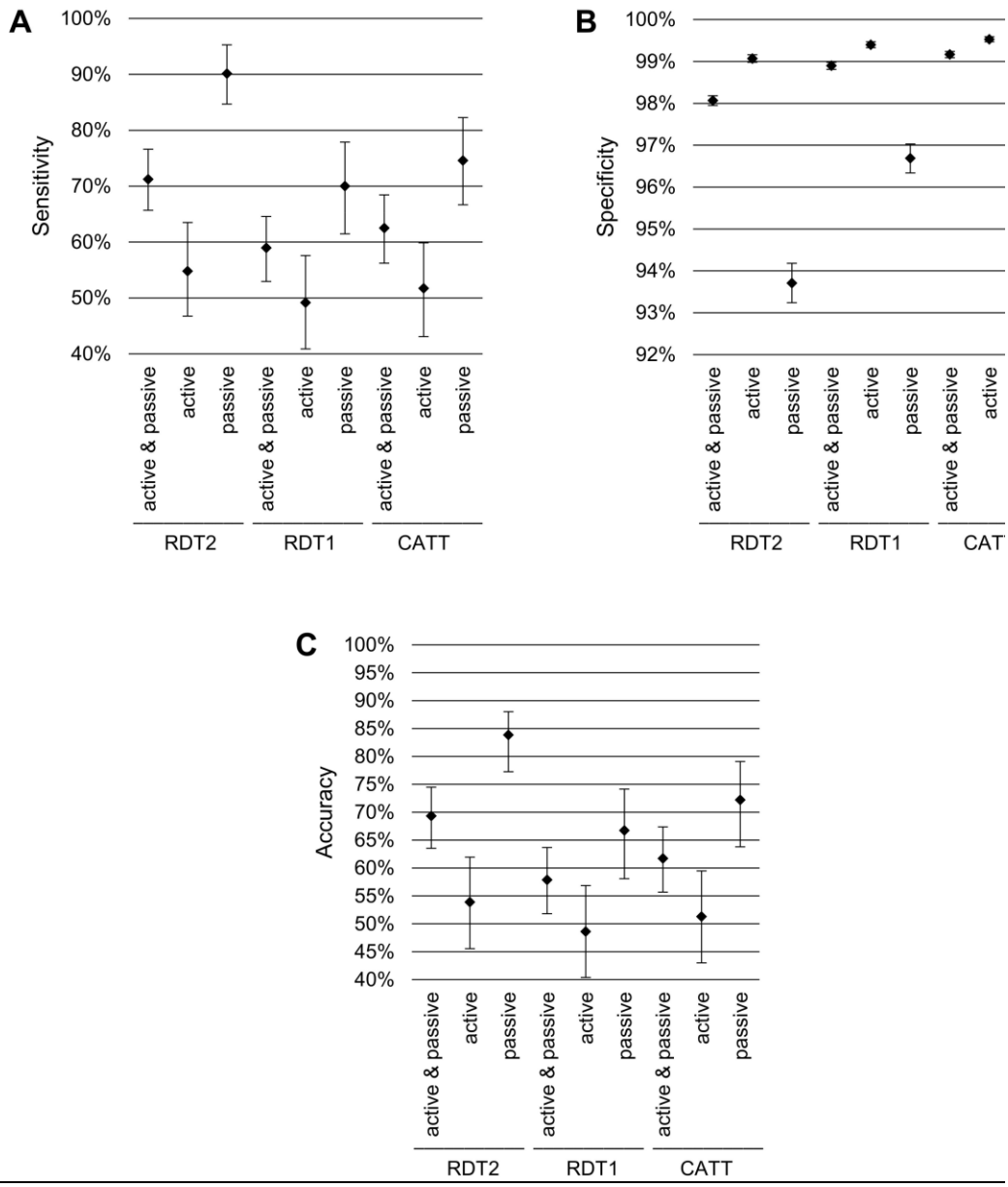
CATT had the best specificity (99.2% [CI: 99.1%; 99.2%]), followed closely by RDT1 (98.9% [CI: 98.8%; 99.0%]) and RDT2 (98.1% [CI: 98.0%; 98.2%]). With all the screening tests, specificity was significantly higher in active than in passive screening. In active screening, specificity was highest with CATT (99.5% [CI: 99.5%; 99.6%]), which was followed by RDT1 (99.4% [CI: 99.3%; 99.5%]) and RDT2 (99.1% [99.0%; 99.2%]). Similarly, in passive screening, specificity was highest with CATT (97.6% [CI: 97.3%; 97.9%]), while lower results were obtained with RDT1 (96.7% [CI: 96.3%; 97.0%]) and RDT2 (93.7% [CI: 93.2%; 94.2%]).

RDT2 had the highest accuracy (69.3% [CI: 63.5%; 74.5%]), followed by CATT (61.7% [CI: 55.7%; 67.4%]) and RDT1 (57.9% [CI: 51.8%-63.7%]). All tests had a higher accuracy in passive than in active screening.

The agreement between the two technicians who read the screening tests was excellent. Cohen's Kappa factor was above 99.8% with all the tests, both in active and passive screening. The differences in sensitivity and specificity between two screening tests are shown in Table 2 for each possible pair of tests. The RDT2 was 12.3% [CI: 3.8%; 20.7%] more sensitive than the RDT1 when the results of active and passive screening were considered together. The difference was particularly pronounced in passive screening, where the sensitivity of RDT2 was 20.1% [CI: 9.4%; 29.8%] higher than that of RDT1. By contrast, there was no evidence of a difference in sensitivity between RDT1 and RDT2 in active screening (+5.6% [CI: -7.4%; 18.7%]). The objective of non-inferiority using a margin

of 8% was met in both active and passive screening. The RDT2 was also more sensitive than CATT (+8.7% [CI: 1.0%; 16.6%]) when the results of active and passive screening were combined, and again, this effect was stronger in passive than in active screening (+15.5% [CI: 7.5%; 24.3%]). There was no evidence of a difference in sensitivity between RDT1 and CATT in both active (-2.6% [CI: -15.2%; 9.5%]) and passive screening (-4.6% [CI: -13.3%; 5.0%]).

The RDT2 was 0.83% less specific [CI: -0.96%; -0.70%] than RDT1 when results of active and passive screening were combined, which was within the non-inferiority margin of 1%. While the difference in specificity was minimal in active screening (-0.33% [CI: -0.44%; -0.23%]), it was more pronounced in passive screening (-2.98% [CI: -3.50%; -2.48%]). The RDT2 was also less specific than CATT (-1.10% [CI: -1.22%; -0.98%]), and this difference



6.2 Figure 2. Sensitivity (A), specificity (B) and accuracy (C) of the RDT2, RDT1 and CATT tests, by screening method.
RDT1: SD BIOLINE HAT rapid diagnostic test; RDT2: SD BIOLINE HAT 2.0 rapid diagnostic test; CATT: card agglutination test for trypanosomiasis
https://doi.org/10.1371/journal.pntd.0006386.g002

was more pronounced in passive (-3.86% [CI: -4.37%; -3.39%]) than in active screening (-0.46% [CI: -0.56%; -0.37%]). The RDT1 was slightly less specific than CATT (-0.27% [CI: -0.37%; -0.17%]), and this difference was also more pronounced in passive (-0.88% [CI: -1.28%; -0.50%]) than in active screening (-0.13% [CI: -0.21%; -0.05%]).

6.2 Table 2. Differences in sensitivity and specificity between screening tests, and by method of screening

Tests compared	Screening method	Difference (%)	
		Sensitivity (95% CI)	Specificity (95% CI)
RDT2−RDT1	Both active and passive	12.3 (3.8;20.7)[#]	-0.83 (-0.96; -0.70) [#]
	Active	5.6 (-7.4;18.7)	-0.33 (-0.44; -0.23) [#]
	Passive	20.1 (9.4;29.8) [#]	-2.98 (-3.50; -2.48) [#]
RDT2−CATT	Both active and passive	8.7 (1.0;16.6) [#]	-1.10 (-1.22; -0.98) [#]
	Active	3.1 (-9.8;15.5)	-0.46 (-0.56; -0.37) [#]
	Passive	15.5 (7.5;24.3) [#]	-3.86 (-4.37; -3.39) [#]
RDT1−CATT	Both active and passive	-3.6 (-11.3;4.4)	-0.27 (-0.37; -0.17) [#]
	Active	-2.6 (-15.2;9.5)	-0.13 (-0.21; -0.05) [#]
	Passive	-4.6 (-13.3;5.0)	-0.88 (-1.28; -0.50) [#]

RDT1: SD BIOLINE HAT rapid diagnostic test; RDT2: SD BIOLINE HAT 2.0 rapid diagnostic test; CATT: card agglutination test for trypanosomiasis.
[#]Difference that is significant at the 5% level.
"RDT2−RDT1" corresponds to the performance of RDT2 minus the performance of RDT1, and likewise for the other pairs of tests.

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Considering that RDT1 and RDT2 are each made using two different antigens, we calculated the sensitivity and specificity of individual antigens. Table 3 shows that for each RDT, individual antigens detected partially overlapping groups of HAT cases, since the sensitivity obtained with single antigens was lower than the result of the RDT. Therefore, having two antigens in these tests resulted in higher sensitivity than if only one antigen had been used. While each of the antigens in RDT2 detected almost the same number of cases and contributed almost equally to the sensitivity of this test, one of the antigens of RDT1 (native VSG LiTat 1.3) detected a larger number of cases than the other antigen (native VSG LiTat 1.5). Similarly, Table 3 shows that the individual antigens of RDT2 contributed almost equally to specificity, while in the case of RDT1, native VSG LiTat 1.3 gave a slightly greater number of false positive results than the other antigen. All antigens were significantly more sensitive in passive than in active screening. The strongest difference was observed with recombinant ISG65 and recombinant VSG LiTat 1.5, whose sensitivity was two times higher in passive than in active screening.

6.3 Table 3. Sensitivity and specificity of individual antigens in screening tests, by screening method

Antigen	Screening method	Sensitivity (95% CI)	Specificity (95% CI)
Recombinant ISG65	Both active and passive	55.0 (49.0;60.9)	98.9 (98.8;99.0)
	Active	37.4 (29.2;45.7)	99.5 (99.4;99.5)
	Passive	74.7 (65.0;82.3)	96.6 (96.3;97.0)
Recombinant VSG LiTat 1.5	Both active and passive	55.3 (49.2;61.3)	98.8 (98.7;98.9)
	Active	37.4 (29.3;45.5)	99.4 (99.4;99.5)
	Passive	75.4 (67.3;82.7)	96.1 (95.7;96.4)
Native VSG LiTat 1.3	Both active and passive	52.7 (46.7;58.6)	99.1 (99.0;99.2)
	Active	40.2 (31.7;48.3)	99.5 (99.5;99.6)
	Passive	66.8 (59.0;74.0)	97.3 (97.1;97.6)
Native VSG LiTat 1.5	Both active and passive	47.0 (40.8;52.8)	99.3 (99.3;99.4)
	Active	38.8 (30.6;47.2)	99.6 (99.5;99.7)
	Passive	56.4 (47.2;65.8)	98.2 (98.0;98.5)
CATT antigen (trypanosomes expressing VSG LiTat 1.3)	Both active and passive	62.5 (56.2;68.4)	99.2 (99.1;99.2)
	Active	51.8 (43.1;59.9)	99.5 (99.5;99.6)
	Passive	74.6 (66.7;82.3)	97.6 (97.3;97.9)

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6.4 Table 4. Sensitivity of the three screening tests and individual antigens, by disease stage

Test	Antigen (s)	Disease stage	Sensitivity (95% CI)
RDT2	Recombinant ISG65 and recombinant VSG LiTat 1.5	Early	59.8 (52.2;67.3)
		Late	87.9 (81.3;93.7)
	Recombinant ISG65	Early	40.5 (32.6;47.9)
		Late	76.6 (67.9;84.5)
	Recombinant VSG LiTat 1.5	Early	38.2 (30.7;45.9)
		Late	80.4 (72.7;87.8)
RDT1	Native VSG LiTat 1.3 and native VSG LiTat 1.5	Early	49.7 (41.9;57.8)
		Late	73.2 (64.4;81.7)
	Native VSG LiTat 1.3	Early	41.9 (34.0;49.4)
		Late	68.9 (60.2;77.5)
	Native VSG LiTat 1.5	Early	34.6 (27.2;42.4)
		Late	65.7 (56.5;74.5)
CATT	Trypanosomes expressing VSG LiTat 1.3	Early	50.6 (42.7;58.8)
		Late	80.2 (72.3;87.8)

RDT1: SD BIOLINE HAT rapid diagnostic test; RDT2: SD BIOLINE HAT 2.0 rapid diagnostic test; CATT: card agglutination test for trypanosomiasis; VSG: variant surface glycoprotein.

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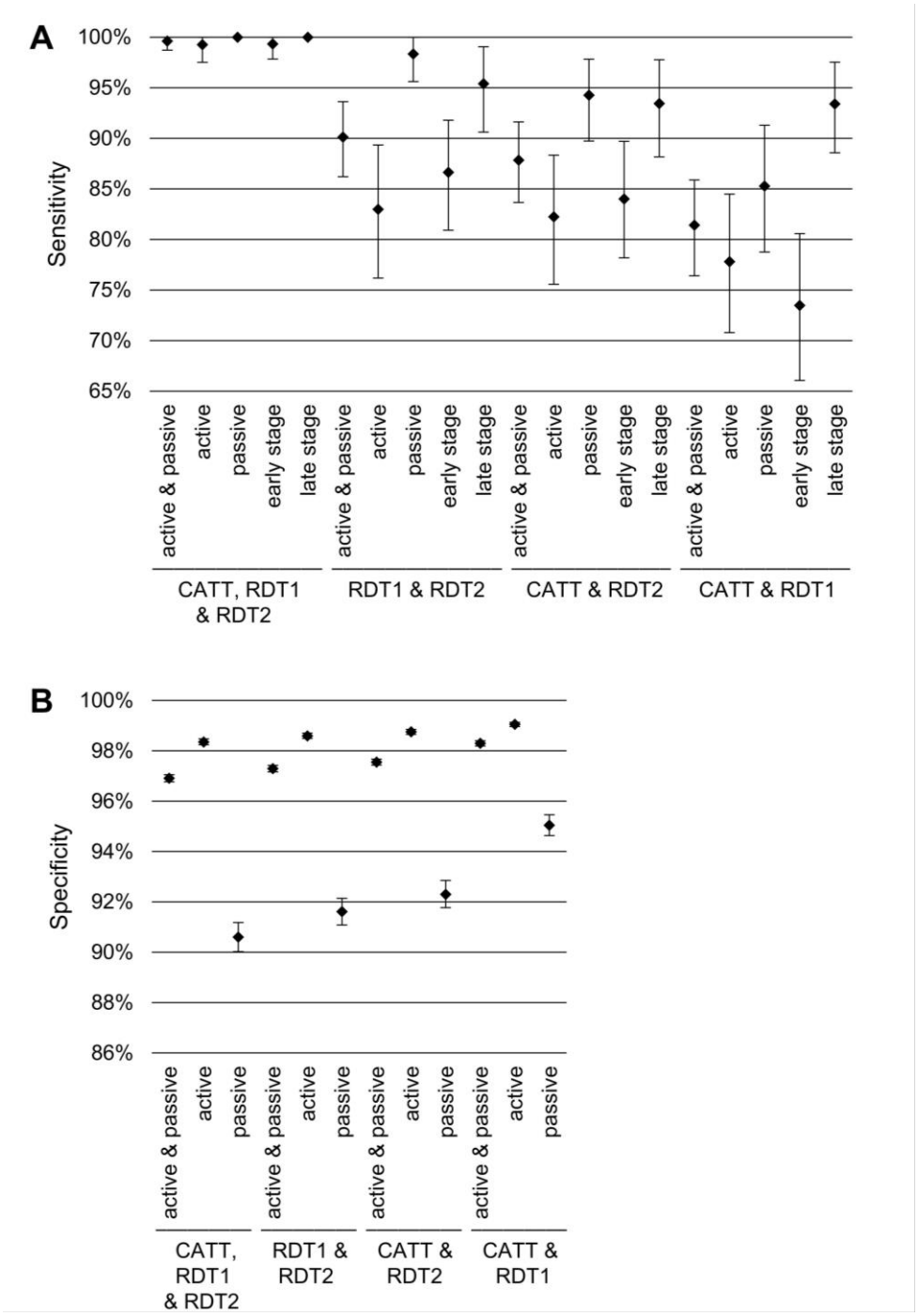
The three screening tests were significantly more sensitive in late stage than in early-stage patients, as shown in Table 4. The strongest difference in sensitivity between stages was observed with CATT, whose sensitivity went up from 50.6% [CI: 42.7%; 58.8%] in early-stage patients to 80.2% [CI: 72.3%; 87.8] in late stage patients. The RDT2 was the most sensitive in both early stage (59.8% [CI: 52.2%; 67.3%]) and late-stage patients (87.9% [81.3%; 93.7%]). Similarly, all the individual RDT antigens were significantly more sensitive in late than in early-stage patients. The largest difference between stages was observed with recombinant VSG LiTat 1.5 (42.1%), while the smallest difference was with native VSG LiTat 1.3 (27.0%). The most sensitive antigen in early-stage

patients was native VSG LiTat 1.3 (41.9% [34.0%; 49.4%]), while the most sensitive antigen in late stage patients was recombinant VSG LiTat 1.5 (80.4% [72.7%; 87.8%]).

We also calculated the diagnostic performance that would be achieved by combining two or three screening tests, with the goal of improving the overall sensitivity of screening, which is important in enhancing control of HAT, as humans are considered the main reservoirs of the disease [21]. The sensitivity and specificity of all possible combinations of two or three screening tests is shown in Fig 3. As expected, the highest sensitivity was achieved by combining all three tests (99.6% [CI: 98.7; 100.0]). This did not reach 100% because there were some differences between the two readers who interpreted test results. The most sensitive combination of two tests was RDT1 and RDT2, which detected 90.1% of cases [CI: 86.2%; 93.6%] and was markedly more sensitive than the individual tests. Lower sensitivity values were obtained by combining CATT and RDT2 (87.8% [CI: 83.7%; 91.6%]) and even more so by combining CATT and RDT1 (81.4% [CI: 76.4; 85.9]). Combining screening tests provided a greater increase in sensitivity in active than in passive screening. In active screening, sensitivity increased from 54.8% [CI: 46.8%; 63.5] with RDT2 to 83.0% [CI: 76.2%; 89.3%] when combining RDT1 and RDT2. In passive screening, this same combination achieved a remarkable sensitivity of 98.4% [CI: 95.6%; 100.0%], compared to 90.1% [CI: 84.7%; 95.3%] with RDT2 alone.



6.3 Figure 3. Sensitivity (A) and specificity (B) of all possible combinations of two or three screening tests, by screening method and by disease stage.

Test combinations are shown in descending order of sensitivity. RDT1: SD BIOLINE HAT rapid diagnostic test; RDT2: SD BIOLINE HAT 2.0 rapid diagnostic test; CATT: card agglutination test for trypanosomiasis. The result of the combination of tests is positive if at least one of the tests is positive, while the result is negative if all the tests of the combination are negative.

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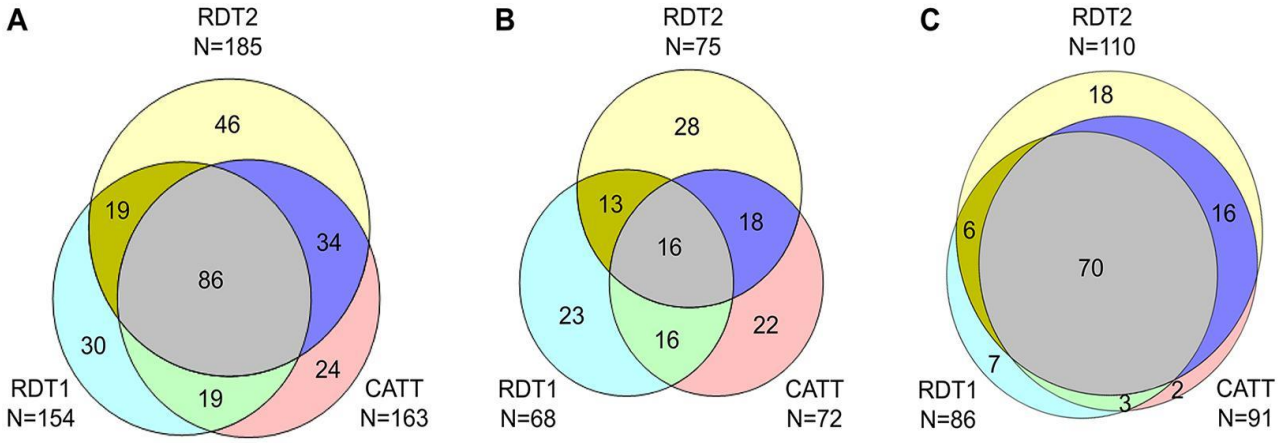
In other words, combining these two RDTs would mean that only 1.6% of cases would have been missed in passive screening, while 9.9% of them would have remained undiagnosed using RDT2 only.



6.4 Figure 4. Venn diagrams showing the number of true positive results obtained with the RDT2, RDT1 and CATT tests.

(A) Results from active and passive screening combined (N = 258 true positives); (B) results from active screening (N = 136 true positives); (C) results from passive screening (N = 122 true positives). For the sake of simplicity, only results obtained by the first reader are shown. The total number of true positives does not equal the total number of cases enrolled in the study (N = 260), as the first reader missed two cases in active screening

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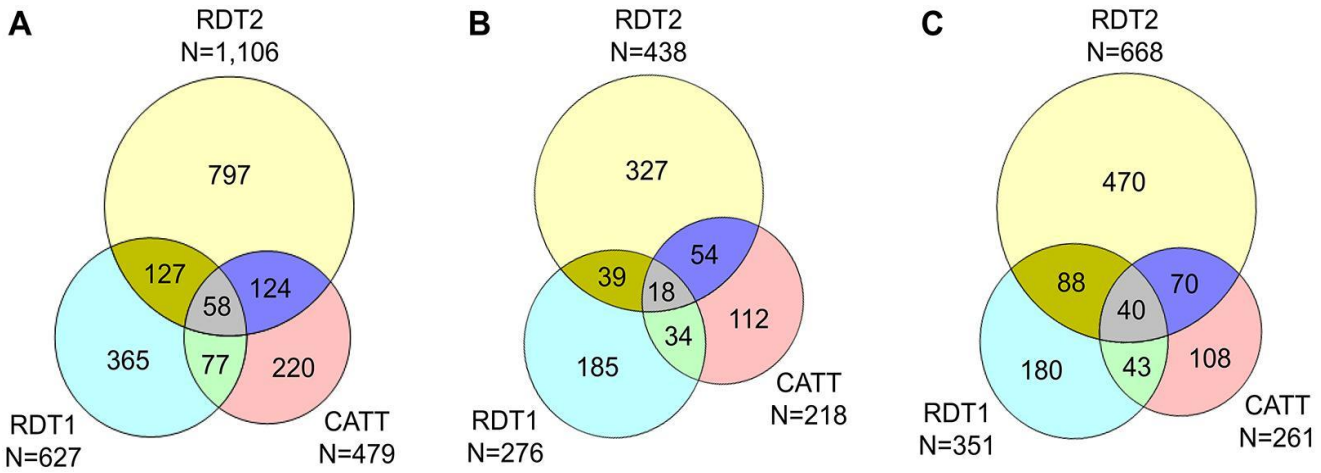
However, using such combinations resulted in some trade-off in specificity, which went down to 96.9% [CI: 96.8%; 97.1%] when the three tests were taken together, or to 97.3% [CI: 97.2%; 97.4%] when combining RDT1 and RDT2.

The contribution of each screening test to the detection of cases and to false positive results is demonstrated using Venn diagrams in Figs 4 and 5. Fig 4 shows that for true positive results, the degree of overlap between the tests was much higher in passive than in active screening. Fig 5 shows that for false positive results, the degree of overlap between the tests was also higher in passive than in active screening, but this difference was much less pronounced than for true positive results.

Both mAECT tests were performed on 124 HAT cases. Ninety cases (72.6%) were positive by mAECT-bc, while only 64 cases (51.6%) were positive by mAECT-wb. There was a high degree of overlap between the two tests, with 58 cases detected using both methods.

The number of HAT cases identified using the different parasitological tests used in the study, as well as the corresponding positivity rates of each of the screening tests, are included as supporting information (S2 Table). This data indicates that among the three parasitological tests performed on blood samples, the positivity rates of screening tests were highest in cases identified by mAECT-bc and lowest in cases diagnosed by CTC. The

positivity rates of screening tests in cases identified by examining lymph node aspirates were not significantly different from the positivity rates obtained in cases that were positive with parasitological tests performed on blood samples. The highest screening test positivity rates were obtained in cases with trypanosomes detected in the cerebrospinal fluid.



6.5 Figure 5. Venn diagrams showing the number of false positive results obtained with the RDT2, RDT1 and CATT tests.
(A)Results from active and passive screening combined (N = 1,768 false positives); (B) results from active screening (N = 769 false positives); (C) results from passive screening (N = 999 false positives). For the sake of simplicity, only results obtained by the first reader are shown.
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Discussion

The main objective of this study, to demonstrate the non-inferiority of the sensitivity and specificity of the RDT2 in comparison to the RDT1, was successfully achieved. However, all three screening tests that were evaluated were unexpectedly insensitive, particularly in active screening, which is in contrast with earlier reports. While CATT has been extensively evaluated and used in clinical settings, and its sensitivity has been reported to range between 68.8% and 100% [22], in this study, the test missed almost half of the cases in active screening. Previous retrospective studies also reported the sensitivity of the RDT1 to be between 82% and 99.6% [8,11]. A sensitivity of 89% was reported in a prospective study of a prototype version of the RDT1 [9], while in another trial, the sensitivity of the commercialized RDT1 was 92% [14].

This apparent discrepancy could be explained by assuming that each of the three screening tests detected cases with different serological profiles, which were only partially overlapping, as evidenced by the results shown in Fig 4. The design of the study, which included three screening tests to identify suspects during enrolment, would be responsible for the low sensitivity of an individual screening test. By contrast, earlier studies only included one, or sometimes two screening tests during enrolment, and as a result, the sensitivity of screening tests could

have been significantly overestimated, since cases with serological profiles that were different from the ones identified by the particular test could have been missed. Therefore, there is the need to explore the possibility of including two or more screening tests in diagnostic algorithms, in order to increase sensitivity and accelerate interruption of disease transmission, particularly by enhancing detection of patients in early-stage disease. Based on the results presented here, strategies combining RDT2 with either RDT1 or CATT in active screening, and combining RDT2 with RDT1 in passive screening, could be considered to enhance case detection. In active screening, each test detected a particularly large number of cases that were missed by the other tests, and combining several screening tests would therefore result in a stronger gain in sensitivity than in passive screening. However, operational aspects would also need to be considered, and cost-effectiveness analyses may provide helpful information to select the most appropriate strategies that would ensure optimal detection of cases. In particular, there is the need to determine whether the extra complexity of the diagnostic algorithm and workload that would result from performing two or more screening tests and having more serological suspects to test by microscopy would cause a significant reduction in the number of people screened by a mobile team in a day, and balance it against the gain in detection of a larger proportion of cases among the people screened. Performing several screening tests would also be a logistical challenge in terms of transportation and storage of tests. Some patients could also refuse to have two or more tests performed on them, an unlikely possibility since blood is taken from the same finger prick. If only one screening test had to be used, the results presented here support using RDT2 in order to enhance case detection, as it was more sensitive than RDT1 and CATT, in both active and passive screening settings. RDT2 was also the most sensitive test in both early and late-stage patients, which indicates that the test is able to detect patients with various clinical profiles. Maximizing sensitivity would be a sensible strategy in a disease elimination context, but the marginally lower specificity of RDT2 would also need to be considered, as it would result in an increase in workload to confirm suspects, decrease in confidence in test results and would also have a negative impact on patients, since a larger number of suspects would need to undergo confirmatory testing, which often requires travelling long distances. Such limitations will become increasingly relevant as progress is being made towards elimination of the disease, since the positive predictive value of screening tests will decline along with the disease prevalence.

Alternatively, investing in the development of a new screening test that would be more sensitive than the tests that were evaluated here, and which would include multiple antigens, could be considered. Such a test might be developed by combining three or more antigens, which could include those in the RDT2, as well as other promising candidates identified in previous studies [23-28]. Other RDTs being developed using recombinant antigens will also need to be considered once they are available and their performance has been evaluated [25]. With the increasing prospects of new, safer treatments for g-HAT that would be effective for both stages of the disease [29,30], a test with high sensitivity and specificity could make a “test and treat” approach possible, without requiring any parasitological confirmation.

A number of hypotheses could be formulated to try and explain the low sensitivity of individual screening tests observed in this study, which would require further investigations. African trypanosomes are notorious for having evolved a mechanism of escaping the host immune system by regularly changing the variant surface glycoprotein (VSG) that composes their cell coat, using a large repertoire of dedicated genes [31,32]. It is therefore likely that HAT patients who have been infected recently could have raised an immune response to only a limited number of VSG antigens, while patients who are in a more advanced disease stage could harbour antibodies against a larger panel of VSGs. This could explain why screening tests that include some specific VSGs, such as the three tests evaluated here, would detect different HAT cases, and why screening tests were more sensitive in late than in early disease stage patients. Similarly, this would provide an explanation for the lower sensitivity that was observed in active screening, since the disease is generally less advanced in most cases among the people screened. Alternatively, the difference in sensitivity between early and late-stage patients could be due to higher antibody titres in the latter because of a longer period of exposure to parasite antigens, and hence stronger immune response. This explanation would better support the finding that the sensitivity of an invariant antigen like ISG65, which is expressed throughout the infection, was higher in late-stage patients. These hypotheses could be tested using animal models infected with *T.b. brucei* [32]. Some patients could have also been infected with trypanosome strains lacking the genes encoding the VSG antigens present in these screening tests. In particular, deletions of the gene encoding VSG LiTat 1.3 have been reported in some *T.b. gambiense* isolates from Cameroon [33], and such deletions could be among the factors responsible for the low sensitivity of CATT and RDT1. Although there is currently no evidence to directly support this hypothesis, it is also conceivable that these deletions could have become increasingly frequent due to the selection pressure applied by the extensive use of CATT in HAT-endemic populations. This phenomenon could have remained unnoticed, since most studies conducted until recently only included CATT during enrolment. Finally, it cannot be excluded that some HAT cases could have corresponded to false positive parasitological test results, which would have been negative with screening tests. It is likely that several of the hypotheses described here could partially explain the observed low sensitivity of screening tests that was found in this study. Other studies comparing the performance of different screening tests in various settings will hopefully help clarify this point.

The fact that RDT1 detected 49 HAT cases that were missed by CATT (Fig 4) could be explained by the presence in RDT1 of the VSG LiTat 1.5 antigen, which is not included in CATT, and also possibly by differences in test formats. On the other hand, it is noteworthy that CATT also detected 58 HAT cases that were missed by RDT1, yet RDT1 contains VSG LiTat 1.3, the antigen that is predominantly expressed by the fixed trypanosomes in the CATT test. This could be due to the nature of the CATT reagents, which in addition to VSG LiTat 1.3, would include other trypanosome antigens that could react with corresponding antibodies in the blood of HAT patients. Another explanation might be the difference in test formats, which may be associated with different binding or exposure characteristics of antigens and epitopes. While in the RDT, antigens are printed on a nitrocellulose

membrane, CATT is performed by mixing a suspension containing fixed parasites with the test sample on a plasticised card. In addition, although the exact composition of the RDT buffer is unknown, it is likely to be different from the CATT buffer (phosphate buffered saline, pH 7.2 with 0.1% sodium azide), which could have an impact on antigenic binding.

In an earlier prospective study that was conducted in the DRC to evaluate the performance of RDT1, the VSG LiTat 1.5 antigen was more sensitive than the VSG LiTat 1.3 antigen (83.6% [CI: 76.3%; 89.0%] and 76.0% [CI: 68.0%; 82.5%], respectively) [14], which is in contrast to what was found in the present study. In another multi-country study that evaluated the performance of the prototype RDT1, identical sensitivity values were reported for each antigen (85.9% [CI: 79.4%; 90.6%]) [9]. While these differences may be due to slightly different study designs, they do not appear to be statistically significant, and would therefore tend to support the view that both antigens contribute equally to the sensitivity of RDT1.

While the three screening tests were highly specific in active screening, they were significantly less specific in passive screening. This difference might be due to serological differences between the two populations, with the population presenting to fixed health facilities being more likely to be infected with other pathogens that could trigger immune responses cross-reacting with the tests. Alternatively, this difference might be explained by the relatively low sensitivity of routine parasitological methods [34]. Indeed, since the HAT prevalence was higher in passive than in active screening, this population was also more likely to have included HAT patients who could have been found positive by screening tests but missed by parasitology, which would have resulted in an underestimate of the specificity of screening tests. The difference in specificity between active and passive screening could thus be an artefact related to the imperfect parasitological reference standard, rather than reflect a real difference in test specificity.

RDT1 and CATT were previously evaluated in another prospective study that was conducted in the DRC [14], which reported that the sensitivity of RDT1 (92.0% [CI: 86.1%-95.5%]) was significantly higher than that of CATT (69.1% [CI: 60.7%-76.4%]). Surprisingly, there was no evidence of any difference in sensitivity between RDT1 and CATT in the present study. The reasons for this discrepancy are unclear, and several hypotheses could be drawn. First, although the studies shared some of the sites, it is possible that the two study populations may have had significantly distinct serological profiles, resulting in different degrees of overlap between the tests. According to this hypothesis, the degree of overlap between screening tests should not be viewed as constant and specific to the tests, but instead, considered as a dynamic phenomenon that may exhibit significant variability in time and in space, depending on the population that is sampled and the underlying immune responses of individual patients. Although this hypothesis seems rather unlikely since the studies were conducted in similar populations, it would be useful to conduct additional studies to establish the reproducibility of such differences. In spite of efforts to ensure compliance with the study protocol and procedures through training, supervision and monitoring,

it is still possible that some of the sites could have performed less well, which could have had an impact on study results. Alternatively, differences between these studies could be due to operational or logistical factors causing some of the tests to have a lower performance than expected. While this seems unlikely, subtle changes during the production of antigens or other components of one of the tests could have occurred and gone undetected, resulting in the lower sensitivity of some test batches.

No failure to follow storage procedures was observed during the study, screening tests were used according to manufacturers' instructions and staff performing the tests ensured that positive and negative controls (for CATT) as well as procedural controls (for RDTs) reacted according to instructions. Yet it is possible that some tests could have deteriorated within the limits of the controls, thereby affecting performance.

The mAECT-bc method [17] may be considered as a replacement of mAECT-wb, which is routinely used in the DRC and other endemic countries. Although based on a subset of participants, the data presented here are in agreement with earlier results showing a significant increase in sensitivity using mAECT-bc. In a first study that was conducted in Guinea, the sensitivity of mAECT-bc was 96.5%, while the sensitivity of mAECT-wb was 78.9% [17]. Another study that was conducted in DRC reported a somehow smaller difference in sensitivity between these two methods (90.9% and 80.4%, respectively) [34]. The lower sensitivity values that were found here (72.6% and 51.6%, respectively) could be explained by the fact that the mAECT methods were only performed on a subset of participants who had been negative with other parasitological methods, and who were therefore likely to include cases with a lower parasitaemia than the other cases that were enrolled in the study. This selection bias could also explain why the difference in sensitivity was higher than in previous reports, since patients with a low parasitaemia could have provided a better dynamic range to evaluate subtle differences in sensitivity. While the difference in sensitivity could be an overestimate of the true difference that would be observed in an unbiased population, implementing the mAECT-bc protocol could be considered to enhance case finding, for a minimal additional workload. Since there was a high degree of overlap between the mAECT-wb and mAECT-bc results, performing both methods may not be justified, as it would increase costs without resulting in any significant increase in sensitivity. Although introducing mAECT-bc would require specific training to prepare buffy coat samples, it did not present a particular challenge during this study, and therefore, implementing it at other sites that are already equipped to perform mAECT-wb should be relatively straightforward.

While mAECT-bc has been shown to be more sensitive than mAECT-wb, mAECT-wb is known to be more sensitive than CTC [6,34]. Thus, the observation that the positivity rates of the screening tests were highest in cases found positive by mAECT-bc and lowest in cases that were positive by CTC could suggest that screening tests would be more sensitive in low-parasitaemia than in high-parasitaemia cases. Although this would need to be further investigated, it would be in agreement with the assumption that patients with a low parasitaemia would have a stronger immune response, which would facilitate their identification using antibody-detection screening

tests. Conversely, patients unable to mount a strong immune response against trypanosomes and therefore more likely to have a high parasitaemia could be more difficult to identify using these screening tests.

This study confronted a number of challenges, which could have somehow impacted the quality of the results. Although study sites were carefully selected based on the available epidemiological data, the HAT prevalence was generally low, making it necessary to enroll patients at 15 different sites. This presented a significant challenge to the study team in terms of coordination, in particular when considering that most of the sites are located in remote, rural areas that were difficult to access. In addition, there was significant turn-over of personnel at some of the sites, requiring additional training. Enrolment was also interrupted at some sites due to stock-outs of supplies, such as mAECT kits. While the study was blinded, it is possible that technicians performing the tests could have been aware of the clinical status of some participants. This is probably more likely to be true in passive screening, since the number of patients presenting daily to health facilities was sometimes very low, making blinding more difficult. Although it is hypothetical, this imperfect blinding could be one of the factors leading to the high degree of overlap of true positive results, and to a lesser extent of false positive results, between the tests that was found in passive screening.

The results presented here have confirmed that the RDT2 would be a useful test for both active and passive screening, either as a single test or in combination with other screening tests. Since it is produced using recombinant antigens exclusively, it will also be easier and safer to manufacture than screening tests that are made with native antigens. The RDT2 is thus a welcome addition to the set of tools that are currently available to control and eventually eliminate HAT.

Supporting information

S1 Fig. **Venn diagrams showing the number of true positive results obtained with the RDT2, RDT1 and CATT tests among early-stage (A) and late-stage (B) cases**. For the sake of simplicity, only results obtained by the first reader are shown. The total number of true positives does not equal the total number of cases enrolled in the study (N = 260), as the first reader missed two cases in active screening.

(TIF)

S1 Table. STARD checklist.

(DOCX)

S2 Table. Results of parasitological tests performed on HAT cases and positivity of screening tests in cases that were positive with specific parasitological tests. CTC: capillary tube centrifugation; mAECT-wb: mini anion exchange centrifugation technique on whole blood; mAECT-bc: mini anion exchange centrifugation technique on buffy coat. (DOCX)

S3 Table. Malaria RDT results obtained in HAT cases and serological suspects. Malaria prevalence values correspond to the percentage of positive malaria RDT results obtained among participants that were tested with a malaria RDT. For the sake of simplicity, only results obtained by the first reader are shown.

(DOCX)

S4 Table. Sensitivity of HAT screening tests in malaria RDT positive and negative participants in passive screening. For the sake of simplicity, only results obtained by the first reader are shown.

(DOCX)

S1 Data. Study database.

(CSV)

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6. Performance evaluation of a prototype rapid diagnostic test for combined detection of *gambiense* human African trypanosomiasis and malaria

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Evaluation of prototype combined rapid diagnostic test for sleeping sickness and malaria

Abstract

Background

Malaria is endemic in all regions where *gambiense* or *rhodesiense* human African trypanosomiasis (HAT) is reported, and both diseases have similarities in their symptomatology. A combined test could be useful for both diseases and would facilitate integration of the screening for *gambiense* HAT (gHAT) and malaria diagnosis. This study aimed to evaluate a combined prototype rapid diagnostic test (RDT) for gHAT and malaria.

Methods

Blood samples were collected in the Democratic Republic of the Congo and in Uganda to evaluate the performance of a prototype HAT/Malaria Combined RDT in comparison to an individual malaria RDT based on *Plasmodium falciparum* (*P.f.*) Histidine Rich Protein II (HRP-II or HRP2) antigen (SD BIOLINE Malaria Ag *P.f.* RDT) for malaria detection and an individual gHAT RDT based on recombinant antigens, the SD BIOLINE HAT 2.0 RDT for HAT screening. Due to the current low prevalence of gHAT in endemic regions, the set of blood samples that were collected was used to evaluate the specificity of the RDTs for gHAT, and additional archived plasma samples were used to complete the evaluation of the HAT/Malaria Combined RDT in comparison to the HAT 2.0 RDT.

Results

Frozen whole blood samples from a total of 486 malaria cases and 239 non-malaria controls, as well as archived plasma samples from 246 gHAT positive and 246 gHAT negative individuals were tested. For malaria, the sensitivity and specificity of the malaria band in the HAT/Malaria Combined RDT were 96.9% (95% CI: 95.0–98.3) and 97.1% (95% CI: 94.1–98.8) respectively. The sensitivity and specificity of the SD BIOLINE malaria Ag *P.f.* RDT were 97.3% (95% CI: 95.5–98.6) and 97.1% (95% CI: 94.1–98.8) respectively. For gHAT, using archived plasma samples, the sensitivity and specificity were respectively 89% (95% CI: 84.4–92.6) and 93.5% (95% CI: 89.7–96.2) with the HAT/Malaria Combined RDT, and 88.2% (95% CI: 83.5–92) and 94.7% (95% CI: 91.1– 97.2) with the HAT 2.0 RDT. Using the whole blood samples that were collected during the study, the specificity of the HAT/Malaria Combined RDT for gHAT was 95.8% (95% CI: 94.3–97.0).

Conclusion

The HAT/Malaria Combined prototype RDT was as accurate as the individual malaria or gHAT RDTs. The HAT/Malaria Combined prototype RDT is therefore suitable for both malaria diagnosis and gHAT screening. However, there is a need to assess its accuracy using fresh samples in prospective clinical trials.

Author summary

The annual number of reported cases of human African trypanosomiasis (HAT), also known as sleeping sickness (SS), is currently below 1,000 cases worldwide. The Democratic Republic of the Congo (DRC), the most affected country, and Uganda, which shares a border with DRC, are both endemic for *gambiense* HAT (gHAT). The main strategy to control gHAT is screening of at-risk individuals, followed by diagnosis and treatment of confirmed cases. However, this strategy and even the passive screening as currently implemented become less efficient with declining incidence, justifying innovative strategies to efficiently detect the remaining cases. All areas where gHAT occurs are also endemic for malaria, presenting an opportunity to integrate gHAT screening activities within malaria control activities. This integration is warranted by the fact that in early disease stage, gHAT patients present with signs and symptoms strikingly similar to those of malaria. In order to use malaria diagnosis as an entry point to screen for gHAT, Standard Diagnostics (SD), Republic of Korea (now Abbott Diagnostics, Korea Inc–ADK) made a Combined prototype RDT for both malaria and gHAT, expected to be as accurate as the individual gHAT and malaria RDTs. In this study, we evaluated the accuracy of the Combined prototype RDT using whole blood samples collected in Uganda and DRC, and archived plasma samples collected in DRC, Angola and Central African Republic. We found that the Combined prototype performs just as well as individual RDTs.

Introduction

Malaria transmission is ongoing in all regions where human African trypanosomiasis (HAT, also known as sleeping sickness), is endemic. However, the opposite is not true [1–4]. This geographic overlap between malaria and HAT (Fig 1) provides a unique opportunity for an integrated control approach for both diseases in the areas where they overlap. Indeed, both diseases need accurate diagnostic tools to guide treatment and control [4–6].

HAT is a vector-borne disease transmitted in sub-Saharan Africa through the bite of several species and subspecies of tsetse flies of the genus *Glossina*. HAT is caused by two subspecies of *Trypanosoma brucei (T.b.)* namely *T.b. gambiense* and *T.b. rhodesiense*. *Trypanosoma b. gambiense* is responsible for a chronic form of the disease, while *T.b. rhodesiense* causes a more acute disease form. *Rhodesiense* HAT (rHAT) is found in eastern and southern Africa. It is a zoonotic disease that only accidentally involves human beings. On the other hand, *gambiense* HAT (gHAT) is endemic in central and western Africa and represents >98% of all HAT cases [4, 7, 8].

Thirty-six African countries have historically been endemic for HAT, of which 24 are endemic for *gambiense* HAT (referred to as HAT or gHAT), and 13 for *rhodesiense* HAT (referred to as rHAT). Uganda is endemic for both forms. Among the 24 countries affected by *T.b. gambiense*, Angola, the Democratic Republic of the Congo (DRC), South Sudan, Chad, the Central African Republic (CAR), the Republic of Congo, Guinea and Uganda represent 98% of all reported gHAT cases [9]. The DRC reported up to 85% of all cases between 2012 and 2016 [8, 10].

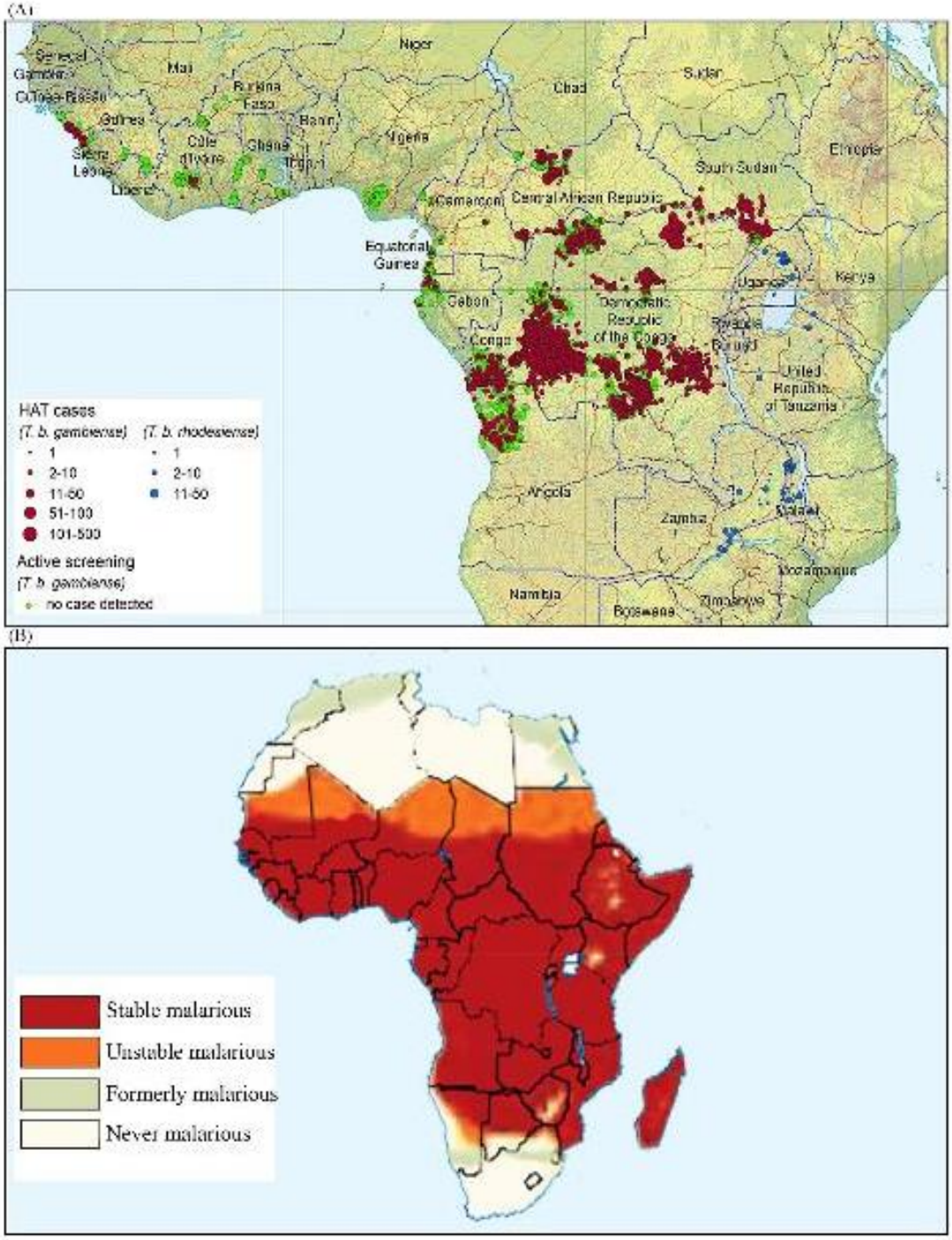
Malaria is a vector-borne disease caused by *Plasmodium* spp. In 2018, a total of 228 million cases of malaria were reported compared to 216 million in 2016. If left untreated, malaria may result in severe complications and lead to death. In 2018, 405,000 deaths due to the disease were reported globally compared to 445,000 in 2016. The African region continues to account for more than 90% of malaria cases and deaths worldwide. DRC is second the most widely affected country in the world, after Nigeria; it accounted for 12% of reported cases and 11% of deaths in 2018 [11, 12].

Patients with malaria or early-stage HAT often present with similar symptoms, such as headache, flu-like symptoms, malaise, joint pains, fever and chills. Diagnostic tests are therefore needed to differentiate these diseases and ensure early HAT treatment to prevent complications, such as irreversible sequelae due to invasion of the central nervous system (CNS) by parasites [4, 5, 9, 14–17]. Delayed diagnosis and treatment of both diseases also promotes further disease transmission. As such, diagnostic tools for both diseases play a key role in control and elimination efforts. Whereas HAT control programs are presently targeting elimination [18, 19], malaria programs in HAT endemic countries target disease control as malaria is still highly endemic in these areas [20]. The substantial decrease in HAT prevalence in recent decades has led to a well-known public health paradox, which is that the few remaining cases and/or carriers are more difficult to identify, cost more money and intervention for HAT control activities become less efficient, with consequences that donors could stop funding for HAT. This

situation hinders elimination and could lead to HAT re-emergence. Therefore, there is a need for innovative and cost-effective strategies to identify the last HAT cases in order to reach and sustain elimination. Such strategies should consider disease spread, preparedness, involvement of health care facilities in the elimination process and opportunities in the overall health care system [2, 21]. As HAT occurs in areas that are highly endemic for malaria, HAT elimination programs could benefit from the well-established and omnipresent malaria control networks by introducing an effective and affordable diagnostic tool for HAT that would be used in an integrated manner for malaria diagnosis. This would allow for a sustained screening for HAT in a subset of the population living in HAT and malaria co-endemic areas.

Today, in addition to microscopy, the diagnostic test that is recommended for malaria in most sub-Saharan African countries is a rapid diagnostic test (RDT) based on *Plasmodium falciparum* (*P.f.*) Histidine Rich Protein II (HRP-II or HRP2) antigen (Ag) [6]. For HAT screening, the most widely used tool has been the Card Agglutination Test for Trypanosomiasis (CATT), which has played an important role in reducing the HAT burden. However, CATT is difficult to implement in peripheral health facilities because of the need for a cold chain for reagent storage, equipment requiring electricity and lack of a single test format [18]. To address these challenges, Standard Diagnostics, Inc. (SD, Geonggido, South-Korea), now Abbott Diagnostics Inc, Korea, and Coris BioConcept (Gembloux, Belgium) have each developed RDTs for HAT screening [22–25]. These tests have been adopted and recommended by the World Health Organization (WHO) [4, 24].

Since areas that are endemic for HAT are also endemic for malaria, both HAT and malaria RDTs could be used on the same patients. Therefore, with support from the Foundation for Innovative New Diagnostics (FIND, Geneva, Switzerland), SD developed a prototype test that combines HAT screening and malaria diagnosis in the same cassette. Screening for HAT is based on antibody detection, and seropositive suspects have to undergo confirmatory testing by microscopy before treatment can be initiated. In contrast, malaria RDTs are based on antigen detection, and a positive result is usually sufficient for a decision to start treatment, although this depends on the national guidelines. A prototype SD BIOLINE HAT/Malaria test was made by combining the SD BIOLINE HAT 2.0 RDT [24] and the SD BIOLINE Malaria Ag *P.f.* RDT. This combined test would be targeted for use in the diagnosis of malaria, with a comparative advantage that screening for HAT is done simultaneously. This would enable detection and treatment of residual cases of HAT during malaria testing, and thus contribute to efforts to prevent re-emergence of HAT and sustain its elimination.



7.1 Figure 1. A) Distribution of human African trypanosomiasis cases reported between 2010 and 2014 [13] and (B) African map of malaria prevalence in 2009 and before (reproduced from "World-map-of-past-and-current-malaria-prevalence-world-development-report-2009.png" file, https://images.app.goo.gl/YYomgUC8fC9ZjTFXA).

https://doi.org/10.1371/journal.pntd.0008168.g001

We report here the accuracy of the SD BIOLINE HAT/Malaria Combined prototype RDT (referred to as Combo RDT) in comparison with HAT and Malaria RDTs in individual formats (referred to as HAT 2.0 RDT and SD Malaria *P.f.* RDT).

Methods

Ethics statement

All diagnostic tests, data entry and data management were conducted according to Standard Operating Procedures (SOP).

The project was carried out in conformity with the Declaration of Helsinki. The sites were appropriate health facilities with staff trained to enrol participants and collect blood samples under good clinical and laboratory practices (GCLP) conditions. The study protocol was approved by the Ethics Committee in DRC (Ethics Committee of the School of Public Health, University of Kinshasa) and in Uganda (The Uganda National Council for Science and Technology). Before inclusion, written informed consent was obtained from participants or legal guardians of minors.

Study design and sites

Malaria assessment. To evaluate the accuracy of malaria diagnosis, samples of whole blood were collected from 7 study sites in the DRC and in Uganda, including 3 sites located in HAT endemic regions and 4 in regions where HAT is not endemic (Fig 2). The 3 study sites in HAT endemic regions were Omugo Health Centre IV in Uganda (Arua district), as well as Masamuna and Masimanimba Hospitals in the DRC (Kwilu province, part of former Bandundu province). The 4 study sites located in regions that are not endemic for HAT were Kasangati Health Centre IV in Uganda (Wakiso district), as well as Bethesda, Virunga and Charité Maternelle Hospitals in the DRC (Nord Kivu province).

HAT assessment. In view of the current low prevalence of HAT in endemic regions, evaluation of the accuracy of the prototype RDT for HAT screening was done on plasma samples that had been obtained from HAT endemic countries (Angola, CAR and DRC) as part of earlier research projects, and archived at Makerere University (Kampala, Uganda). The whole blood samples that were collected during the current study were used to evaluate the specificity of the prototype RDT for HAT.

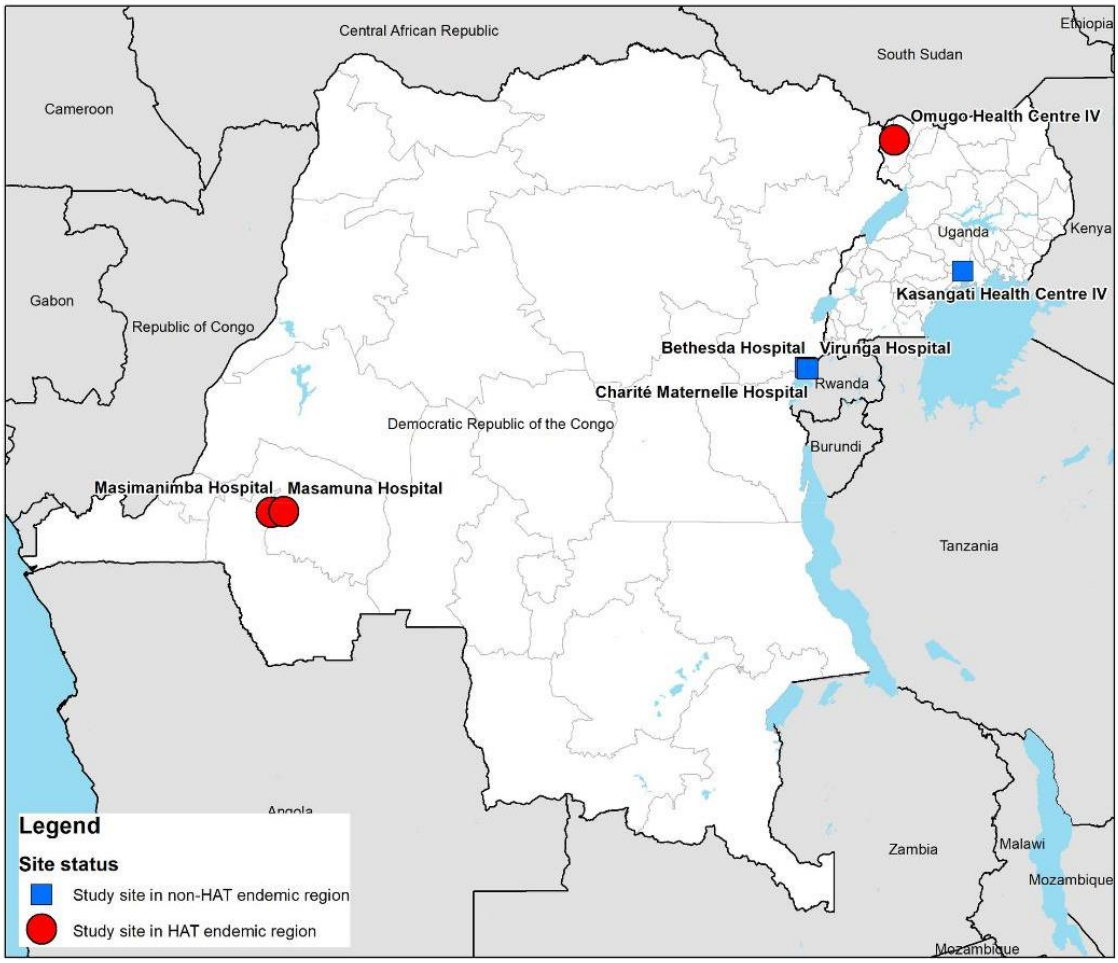
Description of study population

Malaria assessment. Patients presenting themselves at health facilities with symptoms suggestive of malaria or HAT (such as nausea, vomiting, anorexia, flu, weakness, fever, headache, neck pain, body ache, joint pain, itching, sleeping, speech or movement disturbances, weight loss, etc), aged at least 6 months (in DRC) or at least 6 years (in Uganda), and who provided informed consent, were enrolled for sample collection and characterization. Patients

with severe anaemia from whom blood collection was not possible, or whose condition was such that they could not give informed consent were excluded from the study.

Microscopy is currently the common test used as gold standard diagnostic for malaria and is affordable at the level of health facilities. Its shortcomings include the requirement for well-trained and experienced microscopists, rigorous maintenance of equipment, good quality supplies, and that patients with low parasite densities are often missed. Polymerase Chain Reaction (PCR) is more accurate and can detect sub-microscopic malaria infection, but it requires complex equipment, is time consuming and costly [26, 27]. Thus, the reference test (RT) for malaria in this study was microscopy (performed in the field on fresh blood samples) and PCR (performed at Makerere University on stored samples). A sample was considered positive for malaria when it was positive by both microscopy and PCR. It was considered negative if negative by both tests. The sample was excluded if microscopy and PCR results were discordant.

For microscopy, a thick and a thin blood smear were prepared from each participant. The thin smear was fixed with methanol, and all the blood smears stained with 10% Giemsa for 10 minutes. Thick blood smears were examined by microscopy for the presence of asexual parasites and gametocytes. Parasite densities were calculated as described by Adu-Gyasi *et al* (2012) [28] by counting the number of asexual parasites per 200 leucocytes (or per 500, if the count was <10 asexual parasites/200 leucocytes) and adjusting based on the number of white blood cells (WBCs). WBCs were counted manually using a Neubauer haemocytometer. A blood smear was considered negative when examination of 100 fields at examined to determine the parasite species.X1000 did not reveal asexual parasites. In the case of a positive thick smear, the corresponding thin blood smear was examined to determine the parasite species.



7.2 Figure 2. Whole blood sample collection sites
https://doi.org/10.1371/journal.pntd.0008168.g002

Nested PCR was used to detect malaria parasites and to identify the species. Amplification of the 18S rRNA gene was performed using methods and primers described previously [29–31]. It included a primary PCR test using generic primers for *Plasmodium* spp., and a secondary PCR test performed on the product of the primary PCR, using primers specific for *P. falciparum*. The secondary PCR product was visualized on an agarose gel and a qualitative result indicating the presence or absence of a band of the expected size recorded.

HAT assessment. For HAT, stored plasma samples collected in Angola and Central African Republic (CAR) had been pre-selected by CATT, and the ones collected in DRC had been pre-selected by CATT, the SD BIOLINE HAT RDT and/or the SD BIOLINE HAT 2.0 RDT (referred to as HAT RDT and HAT 2.0 RDT respectively) [22–24]. Confirmatory diagnosis of HAT was based on a Composite Reference Standard (CRS), including microscopy of lymph node aspirate, blood examination by the micro-Haematocrit Centrifugation Technique (mHCT) and/or

mini-anion exchange centrifugation technique (mAECT), and CSF examination using the modified single centrifugation (MSC) technique [4]; mAECT was unavailable for CAR evaluations. Samples were considered HAT positive (i.e. from HAT cases) if collected from persons from whom trypanosomes were identified by any of the microscopy methods in the CRS. Plasma samples collected from persons with negative HAT serology and parasitological tests and no history of HAT were considered as controls. Participants enrolled in areas that were not HAT endemic were assumed to be negative for HAT and considered as non-HAT controls.

The whole blood samples that were collected during the current study were preselected using the HAT RDT and HAT 2.0 RDT as screening tests in the DRC, and using the HAT RDT in Uganda. Confirmatory diagnosis of HAT was based on the CRS described above. HAT case and non-HAT control participants were considered as described above.

Sample size. No formal sample size calculations were performed for HAT or malaria assessments.

The target was to collect fresh whole blood samples from 500 malaria case and 500 non-malaria control participants (no target numbers were set for HAT cases or non-HAT control participants). In addition, archived plasma samples from 250 HAT cases and 250 non-HAT controls were used. This was expected to provide information that would be strong enough to evaluate the prototype combo RDT, and inform decisions to start prospective performance evaluation studies in the field.

Whole blood samples collected from malaria cases and non-malaria control participants were used to assess the malaria accuracy and HAT specificity of RDTs, while archived plasma samples were used to assess HAT accuracy.

HAT and malaria RDTs

The SD BIOLINE Malaria Ag *P.f.* test (SD Malaria *P.f.* RDT), the SD BIOLINE HAT 2.0 RDT (HAT 2.0 RDT), and the prototype SD BIOLINE HAT/Malaria Combined test (Combo RDT) are all cassette-format RDTs developed by SD. They include one control band (C band) and one or two test bands for HAT and/or malaria.

The SD Malaria *P.f.* RDT includes one test band for the qualitative detection of the *P. falciparum* HRP-II antigen in human whole blood.

The HAT 2.0 RDT includes 2 test bands to detect antibodies against two trypanosome antigens (recombinant ISG65 and recombinant VSG LiTat 1.5) [24].

The Combo RDT includes two test bands: one to detect the *P. falciparum* HRP-II antigen ("*P.f.*" or "malaria" band) and the other to detect antibodies against two trypanosome antigens (recombinant ISG65 and recombinant VSG LiTat 1.5) ("HAT" band).

Identifiers of proteins cited in this study. *Plasmodium falciparum* histidine rich protein (HRP-II), accession P05227.1 (UniProtKB/Swiss-Prot); *T. brucei* ISG65, accession XP_951587 (GenPept); and *T. brucei* VSG LiTat1.5, accession ABX55936 (GenPept).

Collection and use of fresh whole blood samples

Malaria assessment. To evaluate the accuracy of the RDTs, 4 ml of venous blood were collected by trained staff of the health facility, into tubes containing heparin as anticoagulant. The blood samples were subdivided into two portions and blinded by an independent technician prior to testing. One portion of each sample was used onsite to perform the following tests and procedures: SD BIOLINE Malaria Ag *P.f./Pan* or Ag *P.f.* RDT, microscopy procedures for malaria (regardless of onsite malaria RDT results). From the second portion of blood, 4 aliquots of 500µl each were prepared and frozen in liquid nitrogen and shipped to Makerere University for malaria PCR analysis, and testing with both the SD Malaria *P.f.* RDT and the prototype Combo RDT.

Malaria RDT results obtained on site were used to manage malaria according to the country's guidelines.

HAT assessment. The collected portions of blood described above were also used for HAT assessments as follows:

one portion was tested onsite (2 ml) with HAT RDT and/or HAT 2.0 RDT and subjected to HAT parasitology testing (mHCT and mAECT).

the aliquots shipped to Makerere University were also tested with the prototype Combo RDT (HAT band).

The RDT results obtained onsite were used to guide confirmatory testing by microscopy, according to the HAT diagnostic algorithm.

Evaluation of RDTs at Makerere University

Malaria assessment. Blood samples shipped frozen on dry ice to Makerere University were thawed at room temperature and mixed to ensure homogeneity. All samples were labelled with blinding codes.

To evaluate the accuracy of malaria diagnosis, 5 µl of whole blood was applied to the SD Malaria *P.f.* RDT and to the Combo RDT (malaria band), according to the manufacturer's instructions. The procedure for performing the Combo RDT is the same as that for the SD Malaria *P.f.* RDT, except that the former includes two test lines (one for HAT and another for malaria).

HAT assessment. To evaluate the accuracy of HAT diagnosis, using archived plasma samples, an aliquot of 15 µl of plasma was diluted by adding 15 µl of human blood, freshly collected from a volunteer. This was done in order to ensure that the volume to pipette for RDT testing would not be too small. The sample of whole blood from the volunteer was first checked with each of the 3 RDTs being evaluated, to ensure that it did not give any positive HAT or malaria result. Volumes of 5 µl and 20 µl were then transferred from this 30 µl aliquot and applied to the Combo RDT (HAT band) and to the HAT 2.0 RDT, respectively.

Frozen blood samples were tested with the combo RDT as described above, and the result of HAT band recorded.

Data management and statistical analysis

Participant information and test results at the sample collection sites were recorded on paper case report forms (CRF). The CRFs were transferred to "Programme National de Lutte contre la Trypanosomiase Humaine Africaine" (PNLTHA) in Kinshasa (DRC) or to Makerere University (Uganda) for double data entry using a web-based clinical data management platform (Open Clinica). Data were analysed with Stata SE 15.1 software.

Sensitivity and specificity were calculated for each test as the percentage of positive tests among cases and the percentage of negative tests among controls respectively. Accuracy was assessed by calculating Youden's index [32]. Concordance between different RDTs was also assessed by calculating the corresponding Cohen's kappa factor. We evaluated sensitivity, specificity and Youden's index using 1st reader RDT results, and we assessed the inter-agreement between two independent RDT readers using Cohen's kappa factor.

Positive and negative predictive values (PPV and NPV) were estimated based on the sensitivity and specificity using HAT prevalence values of 0.01%, 0.1%, 1% and 2%. The HAT prevalence in the population through active screening is currently less than 1%. However, in passive screening this proportion could be up to 1% or 2%, especially among patients that would be preselected based on signs and symptoms indicative of HAT. False discovery rate (FDR) and false omission rate (FOR), easy translation of PPV and NPV by a surveillance programme, could be estimated from PPV and NPV as described below. Based on the DRC National Malaria Control Program (Programme National de Lutte contre le Paludisme— PNLP) annual report for 2016, about 70% of malaria suspects tested positive with malaria RDTs among people attending health facilities. We evaluated and reported the PPV and NPV considering a pre-test probability in health facility setting of 70%.

PPV = sensitivity x prevalence / (sensitivity x prevalence) + (1 –specificity) x (1 - prevalence)

NPV = (specificity x (1 –prevalence)) / ((1 –sensitivity) x prevalence) + (specificity x (1 – prevalence))

FDR = 1 –PPV

FOR = 1 –NPV

Results

Malaria

Enrolment. Among 725 participants enrolled, 486 were malaria cases and 239 non-malaria controls. Of the total participants, 459 (including 340 malaria cases) were enrolled in HAT endemic regions, while 266 (146 malaria cases) were from non-HAT regions. The median age was 24 years with an interquartile range (IQR) of 17 years (18–35 years old). The proportion of females was 61.9% (95% CI: 58.3–65.4).

Among the 725 participants enrolled, the number that tested positive was respectively 480 for individual SD malaria *P.f*. RDT, of which 473 were positive with the reference test (RT), 478 positive with the Combo RDT malaria band

(471 positive to RT), 245 negative with the individual SD malaria *P.f.* RDT (232 negative to RT), and 247 negative with the Combo RDT malaria band (232 negative to RT) as shown in Fig 3.

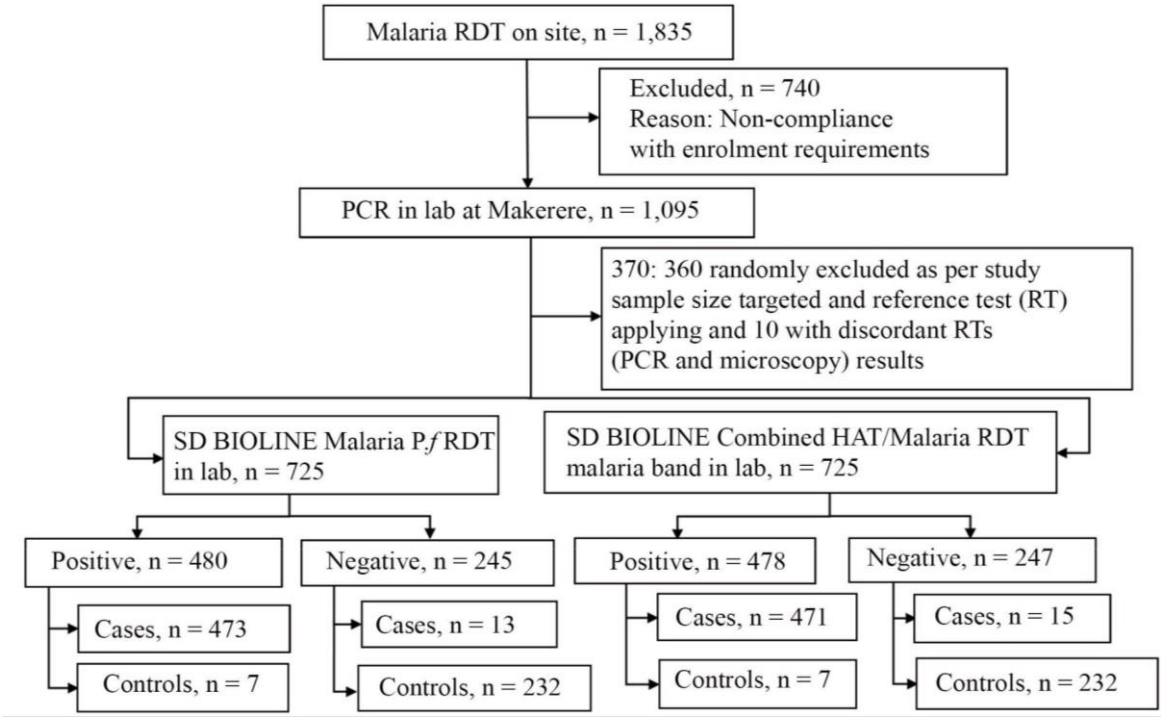
Among the 480 participants who were positive to the individual malaria *P.f.* RDT, 335 were enrolled from HAT endemic regions (333 positive to RT) and 145 (140 positive to RT) from non-HAT regions. Among the 478 participants positive to Combo RDT malaria band, 335 were enrolled from HAT endemic regions (333 positive to RT) and 143 (138 positive to RT) from non-HAT regions.

The number of participants and RDT results related to malaria assessment are detailed in supplemental S1 Table.

RDT sensitivity and specificity. The sensitivity and specificity of the RDTs at each site are shown in Table 1. The sensitivity and specificity of the SD Malaria *P.f.* RDT were 97.3% (95% CI: 95.5–98.6) and 97.1% (95% CI: 94.1–98.8) respectively. For the malaria band of the Combo RDT, the sensitivity was 96.9% (95% CI: 95.0–98.3) and the specificity was 97.1% (95% CI: 94.1–98.8).

The sensitivity of the SD Malaria *P.f.* RDT was of 97.9% (95% CI: 95.8–99.2) in HAT endemic regions, while it was 95.9% (95% CI: 91.3–98.5) in non-HAT endemic regions. The sensitivity of the Combo RDT malaria band was 97.9% (95% CI: 95.8–99.2) in HAT endemic regions and 94.5% (95% CI: 89.5–97.6) in non-HAT endemic regions. The specificity of the SD Malaria *P.f.* RDT was 98.3% (95% CI: 94.1–99.8) and 95.8% (95% CI: 90.5–98.6) in HAT endemic and non-HAT endemic regions respectively, while the specificity of the Combo RDT malaria band was 98.3% (95% CI: 94.1–99.8) and 95.8% (95% CI: 90.5–98.6), respectively.

Concordance between the SD malaria *P.f.* RDT and the Combo RDT malaria band was 99.4%. Inter-reader agreement for SD Malaria *P.f.* RDT and for Combo RDT malaria band was 100.0% and 98.5% respectively.



7.3 Figure 3. Flow of study participants with regard to malaria assessment
https://doi.org/10.1371/journal.pntd.0008168.g003

Test accuracy. Youden's index for accuracy was 0.944 (95% CI: 0.929–0.957) for the individual SD Malaria *P.f.* RDT and 0.940 (95% CI: 0.925–0.953) for the Combo RDT malaria band.

Predictive values and field application. Considering the prevalence of malaria among febrile patients (prior test probability among febrile outpatients) in DRC (70% in 2016), the RDTs' positive predictive values (PPV) were 98.7% (95% CI: 97.4–99.4) for both individual SD malaria *P.f.* RDT and Combo RDT malaria band. The negative predictive values (NPV) were 94% (95% CI: 90.1–96.4) and 93.1% (95% CI: 89.1–95.7) respectively for the SD individual malaria *P.f.* RDT and the Combo RDT malaria band.

7.1 Table 1. Malaria sensitivity and specificity of the RDTs by study site

	SD Malaria *P.f* RDT		Combo RDT malaria band	
Site	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Masamuna	96.6% (91.4–99.1)	98.7% (92.8–100)	96.6% (91.4–99.1)	98.7% (92.8–100)
Masimanimba	97.4% (86.5–99.9)	97.7% (88.0–99.9)	97.4% (86.5–99.9)	97.7% (88.0–99.9)
Charité Maternelle	94.4% (81.3–99.3)	93.3% (85.1–97.8)	94.4% (81.3–99.3)	93.3% (85.1–97.8)
Virunga	100% (85.8–100)	100% (85.8–100)	95.8% (78.9–99.9)	100% (85.8–100)
Bethesda	100% (84.6–100)	100% (83.9–100)	100% (84.6–100)	100% (83.9–100)
Kasangati	93.8% (84.8–98.3)	-	92.2% (82.7–97.4)	-
Omugo	98.9% (96.1–99.9)	-	98.9% (96.1–99.9)	-

https://doi.org/10.1371/journal.pntd.0008168.t001

HAT

Enrolment. The number of participants and RDT results per site related to HAT assessment are detailed in S1 Table. A total of 492 archived samples were used, including 246 HAT cases and 246 non-HAT controls. Among the 246 HAT cases, 217 samples were positive with individual HAT 2.0 RDT and 219 with the Combo RDT HAT band. Among 246 non-HAT controls, 233 were negative with individual HAT 2.0 RDT and 230 with the Combo RDT HAT band (Fig 4).

The frozen blood samples shipped to Makerere for testing were collected from 981 participants successfully enrolled for HAT assessment. Samples were tested with the Combo RDT (HAT band) and not with the HAT 2.0 RDT. Among the 981 participants, 7 were HAT cases and 974 non-HAT controls, 935 tested negative to the Combo RDT HAT band (933 non-HAT controls) and 46 tested positive (41 non-HAT controls) as shown in Fig 5. In order to assess a potential difference in test accuracy depending on HAT endemicity, 599 participants (including 592 non-HAT controls) were enrolled in HAT endemic regions and 382 (all non-HAT controls) in non-HAT regions. The median age of participants successfully enrolled for HAT assessment was 25 years, with an interquartile range (IQR) of 17 years (19–36 years old). The proportion of females was 63.3% (95% CI: 60.2–66.3).

Among the 935 participants that were negative with the Combo RDT HAT band, 560 were enrolled from HAT endemic regions (558 non-HAT controls) and 375 (375 non-HAT controls) from non-HAT regions. Among the 46 participants that were positive with the Combo RDT HAT band, 39 were enrolled from HAT endemic regions (34 non-HAT controls) and 7 (7 non-HAT controls) from non-HAT regions.

RDT sensitivity and specificity. Using archived plasma samples, the sensitivity was 88.2% (95% CI: 83.5–92) and 89.0% (95% CI: 84.4–92.6) for the HAT 2.0 RDT and prototype Combo RDT HAT band respectively. The

specificity was 94.7% (95% CI: 91.1–97.2) and 93.5 (95% CI: 89.7–96.2) for the HAT 2.0 RDT and prototype Combo RDT HAT band, respectively.

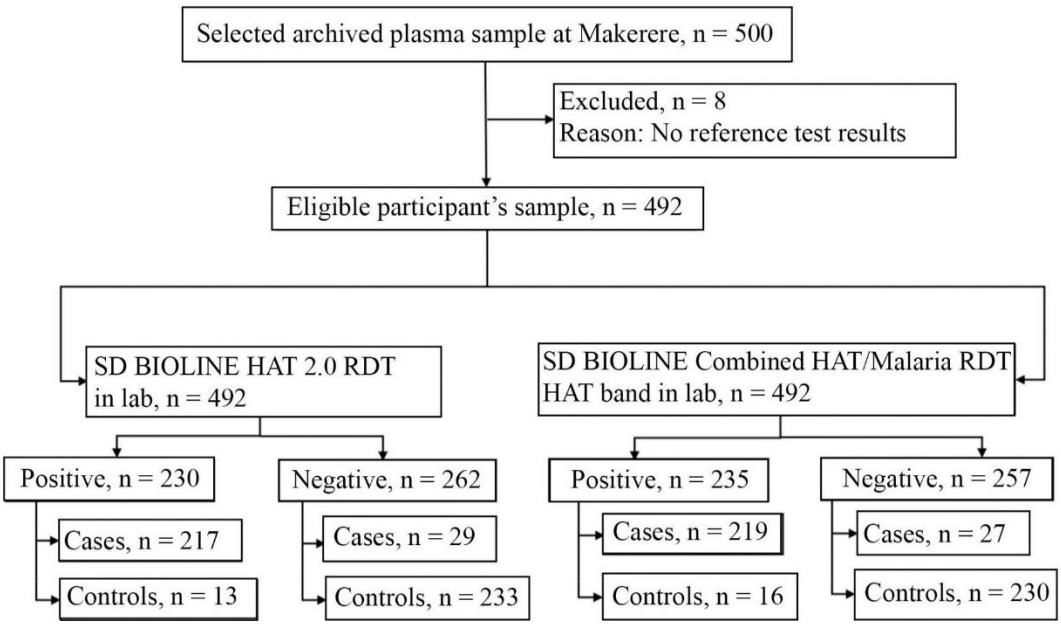
Concordance between the HAT 2.0 RDT and the prototype Combo RDT HAT band was 91.4%.

The inter-reader agreement was 93.9% and 94.7% for the HAT 2.0 RDT and the prototype Combo RDT HAT band, respectively.

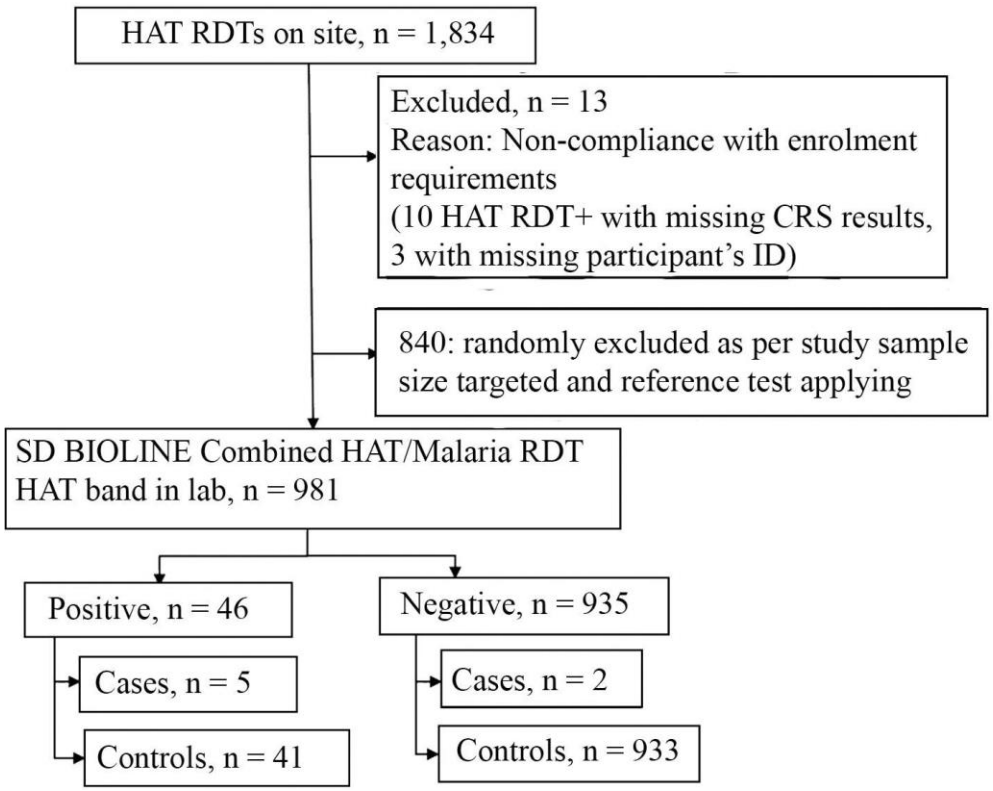
Using frozen blood samples, the overall specificity of the Combo RDT HAT band was 95.8% (95% CI: 94.3–97.0). Its specificity per site is detailed in Table 2 and it was 94.4% (95% CI: 91.9–96.4) in HAT endemic regions and 98.5% (95% CI: 96.3–99.6) in non-HAT endemic regions. The inter-reader agreement was 99.1%.

Test accuracy. Based on archived plasma samples, Youden's index for accuracy was 0.829 (95% CI: 0.776–0.874) for the HAT 2.0 RDT and 0.825 (95% CI: 0.772–0.871) for the Combo RDT HAT band.

Predictive values and field application. The PPV and NPV at 0.01%, 0.1%, 1% and 2% HAT prevalence are detailed in Table 3. Considering the current HAT prevalence of 0.1% in DRC, the RDTs' PPVs were 1.64% and 1.35%, respectively for HAT 2.0 RDT and the Combo RDT HAT band. The RDTs' NPV, at the same HAT prevalence rate, were 100% for both HAT 2.0 RDT and the Combo RDT HAT band.



7.4 Figure 4. Flow of study participant's archived plasma samples with regard to HAT assessment
https://doi.org/10.1371/journal.pntd.0008168.g004



7.5 Figure 5. Flow of study participants with regard to HAT assessment using frozen whole blood samples
https://doi.org/10.1371/journal.pntd.0008168.g005

7.2 Table 2. Combo RDT HAT band specificity per study site

	HAT endemic regions			Non-HAT endemic regions			
Site	**Masamuna**	**Masimanimba**	**Omugo**	**Charité Maternelle**	**Virunga**	**Bethesda**	**Kasangati**
Specificity (CI 95%)	96.8% (93.2–98.8)	93.6% (85.7–97.9)	92.3% (87.5–95.8)	97.4% (92.6–99.5)	97.9% (88.9–99.9)	100% (91.8–100)	100% (94.4–100)

https://doi.org/10.1371/journal.pntd.0008168.t002

7.3 Table 3. The Combo RDT and HAT 2.0 RDT predictive values for screening HAT.

HAT Prevalence	**0.01%**	**0.1%**	**1%**	**2%**
Combined RDT				
PPV (%) (95% CI) 0	.1 (0.1–0.2)	1.4 (0.8–2.2)	12.1 (7.9–18.2)	21.8 (14.8–31)
NPV (%) (95% CI)	100	100	99.9 (99.8–99.9)	99.8 (99.7–99.8)
SD HAT 2.0 RDT				
PPV (%) (95% CI)	0.2 (0.1–0.28)	1.6 (1.0–2.8)	14.4 (9.0–22.3)	25.4 (16.7–36.7)
NPV (%) (95% CI)	100	100	99.9 (99.8–99.9)	99.7 (99.6–99.8)

https://doi.org/10.1371/journal.pntd.0008168.t003

Discussion

The present study demonstrated that the HAT/Malaria Combined RDT prototype was as sensitive and specific as the individual SD BIOLINE malaria and HAT RDTs (SD Malaria *P.f.* RDT and HAT 2.0 RDT) in the detection of malaria and screening for HAT.

The concordance between the SD BIOLINE HAT/Malaria Combined test and the individual SD Malaria *P.f.* RDT and HAT 2.0 RDT was very good.

Malaria RDTs should be selected according to country guidelines, and WHO recommends taking into account the results of the WHO product testing programme for malaria RDTs [6]. The SD Malaria *P.f.* RDT that was used as comparator test in the present study has been consistently ranked among the best-performing RDTs in this programme, which is also in line with the WHO/USAID informal consultation that took place in 1999 and stated that malaria RDTs should have a sensitivity above 95% and a specificity of at least 90%. The performance of the SD malaria *P.f.* RDT used in the current study was in agreement with WHO recommendations [33].

We obtained a PPV of the evaluated SD malaria *P.f.* RDT comparable or higher at similar or lowest malaria prevalence compared to previous studies, while the NPV was higher [26, 34]. The false positive rate found in the current study would result into unwarranted treatment of 1.3 persons out of 100 with either individual SD malaria *P.f.* RDT or Combo RDT malaria band positive results, considering a malaria prevalence of 70% (the prior test probability among febrile outpatients in the DRC in 2016). On the other hand, 6 patients suffering from malaria out of one thousand people would be tested negative to the individual SD malaria *P.f.* RDT and 7 malaria patients out of one thousand people would be tested negative to the Combo RDT malaria band at the same prevalence.

With regard to HAT screening, Lumbala *et al* (2018) reported an overall HAT 2.0 prototype RDT sensitivity and specificity in both active and passive screening settings of 71.2% (95% CI: 65.7–76.6) and 98.1% (95% CI: 98.0–98.2) respectively. The HAT 2.0 prototype RDT sensitivity and specificity during passive screening, in health facility settings was 90.1% (95% CI: 84.7– 95.3) and 93.7% (95% CI: 93.2–94.2) respectively, and 54.8% (95% CI: 46.8–63.5) and 99.1% (95% CI: 99.0–99.2) respectively during active screening by mobile teams [24].

The sensitivity of the HAT 2.0 RDT in the present study was higher than that reported by Lumbala *et al* [24], particularly in active screening (and when combining active and passive screening). This could be explained by the design of the previous study, in which three screening tests were used to identify suspects at the enrolment step (CATT, HAT RDT and HAT 2.0 RDT), while a number of the archived clinical samples used in the current study were collected using one or two screening tests (CATT and/or HAT RDT and/or HAT 2.0 RDT). Using three screening tests in the previous study could have resulted in underestimating the sensitivity of the HAT 2.0 RDT, when cases were only detected by the other screening tests.

The specificity of the HAT 2.0 RDT was significantly lower in the current study than what was reported by Lumbala *et al* (2018), especially in active screening setting (as well as when combining active and passive screening settings). The fact that a number of samples that were used in the current study were collected in CAR without using mAECT, the most sensitive parasitological test for HAT, could have resulted in some HAT cases being missed and wrongly considered as negative in the present study, thus resulting in an underestimate of the RDT specificity.

Due to the relatively low specificity, the false positive rate tends to be high, especially now that HAT prevalence has decreased significantly, especially in active screening settings. With 0.1% HAT prevalence, at least 98 people out of 100 screened positive to HAT 2.0 RDT or Combo RDT HAT band would be false positive. At 1% HAT prevalence, only 14 persons out of 100 screened positive to HAT 2.0 RDT and 12 persons out of 100 screened positive to Combo RDT HAT band would be confirmed as HAT cases. Currently, a HAT prevalence of 1% can only be achieved in passive screening, and rarely in active screening. On the other hand, for every 1,000 negative screening results, 1 and 2 would be false negatives (missed cases) with Combo RDT HAT band, compared to 1 and 3 false negatives with HAT 2.0 RDT respectively at 1% and 2% HAT prevalence. As the PPV is declining rapidly with current prevalence and the progress towards HAT elimination, this will result in an increase in workload to confirm suspects, with a negative impact on health workers' and patients' motivation and confidence in positive test results, which would end up in missing true HAT cases. The combo RDT is intended to be used primarily for malaria testing and particularly by malaria programs in HAT endemic settings. But based on the PPV at the current HAT prevalence, it seems that while integration of HAT screening in the malaria control program is theoretically meant to increase the number of HAT tests done and therefore increase the proportion of HAT cases detected, the low PPV might quickly have a negative impact on use of the combo RDT. Indeed, introduction of the combo RDT may lead to HAT testing in sites where the HAT prevalence is even lower than 0.1% (0.01% being the actual cut-off for elimination of HAT as a public health problem). The steady decline in HAT prevalence is leading to a decrease in the positive predictive value, while all persons identified as HAT serological suspects need parasitological confirmation by dedicated personnel. In the context of being confronted with a vast majority of false positives, logistic and financial limitations to perform parasitological HAT confirmation, there is a risk that malaria program health personnel will quickly reject the Combo RDT and return to a single malaria test. This underlines the potential shortcoming of the Combo RDT. To prevent this, it will be necessary to improve the PPV of HAT screening RDTs by for instance increasing the pre-test probability by restraining the use of the Combo RDTs to patients with clinical signs that are more specific of HAT, or using an algorithm that includes more specific molecular or serological tests. As test line positivity for malaria (antigen detection) and HAT (antibody detection) may have a different meaning, in particular for treatment decision, training, follow-up and supervision will be very important prior to introduction of the combo RDT in health facilities and / or its integration in malaria programs.

Limitations of this study

The current study had two main limitations that prevent further analysis and interpretation of results. First, information related to possible malaria treatment up to 42 days prior to blood collection was not available. False positivity following a successful malaria treatment would result in an underestimated RDT specificity. The fact that this information was not collected limits comparison to other study findings, especially in regard of malaria RDT specificity.

Second, due to the continued decline in HAT prevalence, it was not possible to evaluate RDT sensitivity using freshly collected blood, but only using archived plasma. The archived plasma samples had been pre-selected using various screening tests, and some of the samples were rather old (2008) albeit well stored in -80˚C freezers or in liquid nitrogen. This could have had an impact on our results and would therefore make comparisons with other studies difficult. However, the fact that the current study aimed to compare the performance of RDTs using the same samples and in the same testing conditions still makes our findings relevant.

Finally, the fresh blood samples that were collected and shipped frozen to Makerere University were not tested with the HAT 2.0 RDT. It was therefore not possible to compare the HAT specificity of RDTs.

Conclusion

This study has demonstrated equivalence in sensitivity and specificity of a prototype HAT/ Malaria Combined RDT for both HAT and malaria, in comparison to the individual HAT and malaria RDTs.

The HAT/Malaria Combined RDT could be used to enable an integrated approach combining malaria diagnosis and HAT screening in *gambiense* HAT endemic areas. A similar strategy would be a good solution to the increasing problem of under-detection of *rhodesiense* HAT (rHAT), but a rapid diagnostic test for rHAT has so far not been developed. However, this evaluation was performed in an academic laboratory setting which differs from field conditions. Also, testing was done on frozen and/or archived samples, which may have slightly different results to those that would be obtained using fresh blood samples. Thus, there is need to conduct prospective clinical trials to confirm the performance of the HAT/Malaria Combined test under real field conditions and assess the accuracy in the local laboratory at the level of health facilities.

Supporting information

S1 Checklist. STARD checklist.
(PDF)

S1 Table. Number of samples and results of malaria assessment per site and per test.
(DOCX)

S2 Table. Number of samples and results of HAT assessment per site and per test.

(DOCX)

S1 Data. Combo RDT HAT and malaria assessment using frozen whole blood sample data base.
(CSV)

S2 Data. Combo RDT HAT assessment using archived plasma sample data base.
(CSV)

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7. Development and implementation of a strategy for intensified screening for *gambiense* human African trypanosomiasis in Kongo Central province, DRC

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Abstract

Background

The Democratic Republic of the Congo (DRC) accounts for the majority of the reported *gambiense* human African trypanosomiasis (HAT) cases. Kongo Central province in the DRC reports a relatively low, yet steady number of cases, and forms a transboundary focus with Angola and the Republic of Congo. This paper describes an intervention aimed at reducing the case burden in Kongo Central by improving passive case detection, complemented with reactive screening.

Methodology/Principal findings

At the initiation of this programme in August 2015, 620 health facilities were identified and equipped with Rapid Diagnostic Tests (RDTs) for HAT screening. Of these, 603 (97%) reported use of RDTs, and 584 (94%) that continued to use RDTs to the last quarter of 2016 were used in the analysis going forward. Among all health facilities involved, 23 were equipped to confirm HAT by microscopy, and 4 of the latter were equipped to perform molecular testing with loop-mediated isothermal amplification (LAMP). Patients clinically suspected of HAT were tested with an RDT and those with a positive RDT result were referred to the nearest microscopy facility for confirmatory testing. If RDT positive patients were negative by microscopy, they were tested by LAMP, either on fresh blood or blood that was dried on filter paper and transported to a facility performing LAMP. This network of diagnostic facilities reduced the median distance for a patient to travel to a screening facility from 13.7km when the classical card agglutination test for trypanosomiasis (CATT) was used as a screening test in the past, to 3.4km. As a consequence, passive case detection was improved by between 30% and 130% compared to the period before. Furthermore, the proportion of HAT cases detected in early-stage disease by passive screening increased from 27% to 64%

Reactive screening took place in 20 villages where cases were reported by passive screening, and in 45 villages in the neighbourhood of these villages. Reactive screening was responsible for detection of 40% of cases, of which, 90% were in first stage of the disease.

Conclusions

This programme has demonstrated that it is possible to deploy passive screening for HAT at sub-country or country levels in the DRC, and this is made more effective when supplemented with reactive screening. Results and achievements showed an increase in the number of HAT cases detected, the majority of them in early disease, demonstrating that this strategy enables better population coverage and early detection of cases, which is critical in removing the HAT reservoir and interrupting transmission, and could contribute to HAT elimination in regions where it is implemented.

Author summary

A number of diagnostic tests for HAT have recently been developed, to improve case detection. We report on the use of these technologies in a strategy to increase coverage and early detection of HAT cases in Kongo Central province of DRC.

All 620 health facilities in the focus were equipped with RDTs to test patients presenting with symptoms suggestive of HAT. Among these health facilities, 23 were upgraded to perform confirmatory testing, for a final diagnosis. All 620 health facilities in the focus were equipped with RDTs to test patients presenting with symptoms suggestive of HAT. Among these health facilities, 23 were upgraded to perform confirmatory testing, for a final diagnosis.

This strategy has reduced the distance a patient travels to a facility screening for HAT, from 13.7km to 3.4km. From August 2015 to December 2016, the proportion of HAT cases detected, adjusted annually, increased by between 30% and 130% compared to the previous two years, and 64% of them were in early-stage disease, compared to 27% previously. This strategy has enabled better population coverage, and when supplemented with reactive screening, the identification of local outbreaks and early detection of most cases, which is critical in removing the HAT reservoir and interrupting transmission, thus contributing to elimination of the disease.

Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a parasitic disease transmitted by the bite of an infected tsetse fly (*Glossina spp*). The disease is endemic in sub-Saharan Africa, within the limits of the geographic distribution of the tsetse fly. The disease is caused by protozoan parasites belonging to the species *Trypanosoma brucei (T.b.)*. The majority of cases of HAT are caused by the sub-species *T.b. gambiense*, which accounts for all cases reported in West and Central Africa [1]. In many HAT endemic areas, no vector control is carried out, and control of HAT relies on the detection and treatment of infected individuals. This is made difficult by the requirement for case confirmation through visualisation of the parasites in body fluids by microscopy, which in itself has imperfect sensitivity.

In recent years, a number of new technologies have been developed that overcome many of these challenges, including rapid diagnostic tests (RDTs) [2–5] and Light emitting diode (LED) fluorescence microscopy (FM) [6, 7], and in part, the imperfect sensitivity of confirmatory testing has been improved by loop-mediated isothermal amplification (LAMP) of DNA [8, 9]. RDTs do not require electricity to perform, and they can be deployed in any health facility where staff are familiar with use of RDTs, while LED FM has low requirements for electricity, and can be powered by solar panels. These new technologies, in combination with other existing tools, has enabled implementation of a new diagnostic algorithm for HAT, in which clinically suspected patients are first screened with RDTs, and if positive, or if they have clinical signs strongly suggestive of HAT, they are referred to a facility with the capacity for confirmatory testing. Those that are negative with the confirmatory test are subjected to LAMP on fresh blood in sites upgraded to perform LAMP, or on blood samples dried on filter paper collected in confirmatory sites that are not equipped to perform LAMP. At the commencement of the project in August 2015, this algorithm had been implemented successfully in Uganda for two years [10].

While the Democratic Republic of the Congo (DRC) has always reported the greatest case burden annually, prior to, and since initiation of this programme, the number of cases reported in the country has been falling [11]. Active screening by mobile teams moving to HAT endemic villages has until recently been the main strategy to control HAT in DRC. From 2000 to 2012, an average of 90% (ranging from 72% to 95%) of the population in endemic areas were actively screened. The proportion of HAT cases detected by active screening was almost equal to the one detected through passive screening in health facilities, averaging 53%, and a range of 47% to 63%. However, majority of the cases have been diagnosed in early stage through active screening by mobile teams, with the proportion ranging from 62% to 83% and averaging 76%. This could be explained by different challenges faced by passive case detection, such as the fact that people present to health facilities after having persistent signs and symptoms of HAT and/or after visiting different health facilities over lengthy periods of time. In addition, the tests used for screening required electricity and a cold chain, limiting expansion

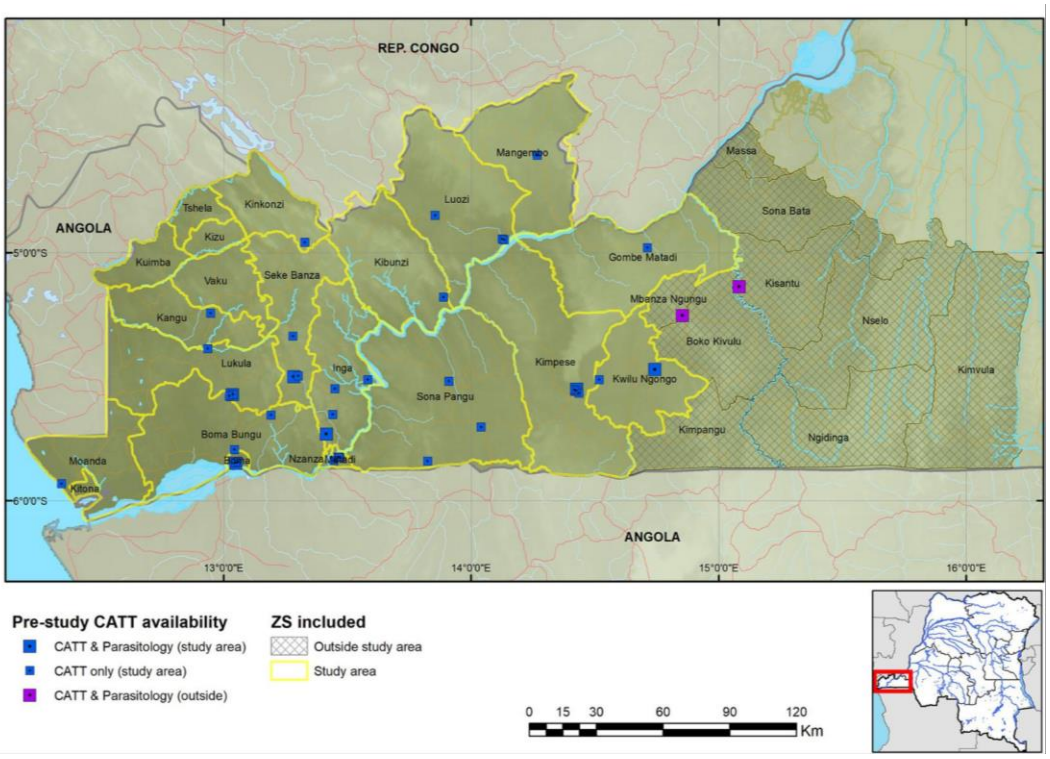
of the diagnostic capacity to available health facilities. [11–14]. Different challenges associated with passive case detection limited passive screening in Kongo Central province to 40 facilities within the study area (of which seven could perform the capillary tube centrifugation technique—CTC and/or the mini-anion exchange centrifugation technique—mAECT, the most sensitive confirmatory parasitological tests for HAT) and two smaller facilities that were outside the study area, where no HAT case was diagnosed for over 5 years prior to the intervention, since 2013 (Fig 1 & S1 Data).

Among the HAT endemic regions in the DRC, the focus in Kongo Central province, formerly Bas Congo, is a transboundary focus bordering endemic areas in the Republic of Congo to the north and Angola to the south. Furthermore, the number of cases reported in Kongo Central in the years before the project had been low–typically between 100 and 200 cases per year, which is relatively few in the context of DRC (Table 1). The reported low number of cases in this HAT focus indicates that a sustained drive to diagnose cases could lead to local elimination of the disease in the region. Furthermore, the province is also one of the smaller ones in the DRC.

In this paper, we report on a programme to improve the coverage of HAT case detection in Kongo Central through an expansion of passive screening to include all health facilities in the Kongo Central HAT focus, complemented by targeted reactive screening in sampled villages that report cases. Success was measured by the number of HAT cases reported, where they were screened, and the disease stage those cases were in. The programme was initiated in August 2015 and implemented in all health facilities in the selected region by November 2015.

Methods

The core phases of the study involved the development of a diagnostic algorithm, identification and characterisation of health facilities in the province, the selection of those that were suitable



8.1 Figure 1. Map of sites performing CATT in Kongo Central province during 2013 and 2014.
The geodata layers were obtained from CC-BY License compatible sources: The CGIAR SRTM 90m datasets (https://bigdata.cgiar.org/srtm-90m-digital-elevation-database/). Further geodata were downloaded from Digital Chart of the World through Diva-GIS (https://www.diva-gis.org/gdata) and OpenStreetMaps (http://www.openstreetmap.org) and rendered by the authors using ESRI ArcGIS following the PLOS guidelines (https://journals.plos.org/plosone/s/figures) or were supplied by PNLTHA.
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for upgrading to perform parasitological techniques and those that were suitable for LAMP. The strategies for monitoring activities and identifying villages in which reactive screening was to be performed were also established.

Health facility characterization

Characterization of health facilities was a process of gathering as much information about the facility using a questionnaire, and its location using a hand-held GPS instrument (S1 Text). The questionnaire, which was completed by the health facility manager, was brief and comprised of questions on the current staffing and laboratory capacity of the facility, as well as the history of diagnosing HAT. A member of the national HAT control programme visited each health zone and trained local extension nurses in facility characterization and use of the GPS. The nurses would then visit each facility and carry out the characterization. The data would subsequently undergo quality control and any adjustments made. These data were then mapped (Fig 1).

8.1 Table 1. Number of HAT cases reported in various provinces (historical) in the DRC from 2010 to 2015

Former province	Number of HAT cases					
	2010	2011	2012	2013	2014	2015
Bas Congo (Kongo Central)	167	105	107	130	70	83
Bandundu	2,925	2,506	3,167	2,415	1,885	1,575
Equateur	308	321	197	71	75	47
Kasai Occidental	270	356	171	132	98	118
Kasai Oriental	697	698	701	502	313	266
Katanga	52	62	91	133	64	49
Kinshasa	153	143	134	166	103	83
Maniema	71	53	128	104	63	57
Province Orientale	986	1,351	1,282	1,971	535	71
Other	0	0	0	0	0	0
Total	5,629	5,595	5,978	5,624	3,206	2,349

Data from Lumbala et al, 2015 [11].

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Upgrading of health facilities

The health facility characterization data were analysed to optimally deploy resources for upgrading facilities to perform microscopy and LAMP. Based on the information gathered, 19 facilities were upgraded to perform microscopy (LED FM sites) and 4 to perform microscopy and LAMP (LAMP sites). The following criteria were considered in identifying the facilities to be upgraded:

- The current HAT diagnostic capacity and the history of diagnosing HAT.
- The laboratory and human resource capacity. Microscopy requires a lab with trained staff,
- and electricity can be provided by installation of solar panels. LAMP requires highly trained staff and a reliable source of electricity.
- The numbers of HAT cases reported in the locality.

All staff involved in the programme received training in clinical diagnosis of HAT, use of the tests, and the procedures for referral, depending on activities implemented in the facility.

Diagnostic algorithm

The diagnostic algorithm for passive screening adopted in Kongo Central was similar to the one described in Wamboga et al., (2017), and is shown in S1 Fig "The *T. b. gambiense* human African trypanosomiasis (gHAT) diagnostic workflow implemented in Kongo Central". Briefly, patients presenting themselves at health facilities with clinical signs indicative of HAT, who test negative with malaria RDTs, since malaria presents similar signs and symptoms, are screened using a HAT RDT, the second level screening for HAT following clinical screening based on symptomatology. Those that are positive with the HAT RDT are tested by microscopy in an algorithm comprising palpation and puncture of lymph nodes (LN) if enlarged, and examination of the aspirate, LED FM on thick smears, CTC, LED FM on thin smears of lysed blood, and mAECT on buffy coat (mAECT-bc). If patients test positive with the HAT RDT at facilities that perform only HAT RDTs (RDT sites) they are referred to facilities equipped to perform microscopy (microscopy or LED FM site). If positive with a confirmatory test, the patient's stage of disease is determined and treatment carried out according to national guidelines. If negative, samples of whole blood and buffy coat are dried onto a filter paper and transported using a programme motorcycle to the nearest LAMP facility (LAMP site) for testing. If a patient is HAT RDT positive and microscopy negative at a facility with the capacity to perform LAMP, this test is performed on fresh samples. If either sample is positive, then the patient is recalled for further testing by microscopy, and if negative, no further testing is undertaken, but the patient is asked to present in three months for repeat testing with an RDT.

The RDTs used in this programme were the SD BIOLINE Malaria Antigen *Plasmodium falciparum* test (SD Malaria *P.f.* RDT) for malaria diagnosis and the SD BIOLINE HAT RDT (HAT RDT) for HAT screening. Both are cassette-format RDTs developed by Standard Diagnostics, Inc. (SD, Geonggido, South-Korea), now Abbott Diagnostics, Korea Inc–ADK, Republic of Korea.

The Malaria *P.f.* RDT includes one test band for the qualitative detection of the *P. falciparum* Histidine Rich Protein II (HRP-II or HRP2) antigen (Ag) [15] in human whole blood. The SD BIOLINE HAT RDT includes two test bands to detect antibodies against two trypanosome variable surface glycoprotein (VSG) antigens (LiTat 1.3 and LiTat 1.5) [2, 3].

During reactive screening, patients were screened using the card agglutination test for trypanosomiasis (CATT) or CATT and RDTs in parallel, and if either was positive, then the patient underwent a series of microscopy tests in the following order: LN-CTC-mAECT. Patients found positive using this algorithm were staged and referred for treatment. Suspects that were negative had samples of whole blood and buffy coat dried on filter

paper and transported to a facility performing LAMP. Those positive by LAMP were recalled for further testing by microscopy.

Monitoring of activities

Activities were monitored through a combination of monthly reporting by all RDT facilities by SMS, phone call or email to the local coordinator, on the number of patients tested and numbers positive. Facilities performing microscopy and LAMP also submitted details on tests performed, their results, disease stage and village of origin of any cases. These data were analysed and mapped as they came in.

Reactive screening

In villages from which cases were identified by passive screening, a follow up reactive screening was scheduled. At a date in the following months (when logistically possible) a mobile team using either a vehicle or motorbikes (mini-mobile team) visited a number of sampled and neighbouring villages and screened all persons that presented themselves. This followed the national guidelines [16] but used the diagnostic algorithm described above, and was done using either CATT or both CATT and RDTs in parallel.

Metrics of activities' results

To drive elimination of HAT, it is necessary to maximise the number of people screened among the at-risk population, in efforts to identify most cases early during the course of infection [17, 18]. Thus, success of the programme is primarily measured through comparison of the number of cases identified by passive screening before, versus during the programme, and by analysis of the proportions of these cases that were in the early stages of HAT. Further metrics include the coverage, the HAT RDTs usage at different facility levels, and the proportions of serological suspects from RDT facilities that were successfully referred for confirmation.

The coverage was evaluated in terms of distance to the health facility, and of the population within a 5-8kms radius around sites. This was evaluated by using data extracted from the WorldPop gridded population data sets and overlaying the raster layer of the distances to health facilities [19]. According to DRC health policies, a health facility should cover a population in a radius of 5-8kms [20]. The coverage is the percentage of the population within 5-8kms radius of each of the health facilities that implemented the project and screened patients using HAT RDTs, over the total population in the selected region.

Ethical considerations

This project was carried out in conformity with the Declaration of Helsinki, and was approved by the Ethics Committee of the School of Public Health, University of Kinshasa, DRC (Letter Ref. No ESP/CE/049/14, October 02nd, 2014). Clinical use of HAT RDTs in DRC was approved in 2014 (Letter Ref. No. MS.1251/SG/THA/774/MK/2014, from the General Secretary, Ministry of Public Health). Management of

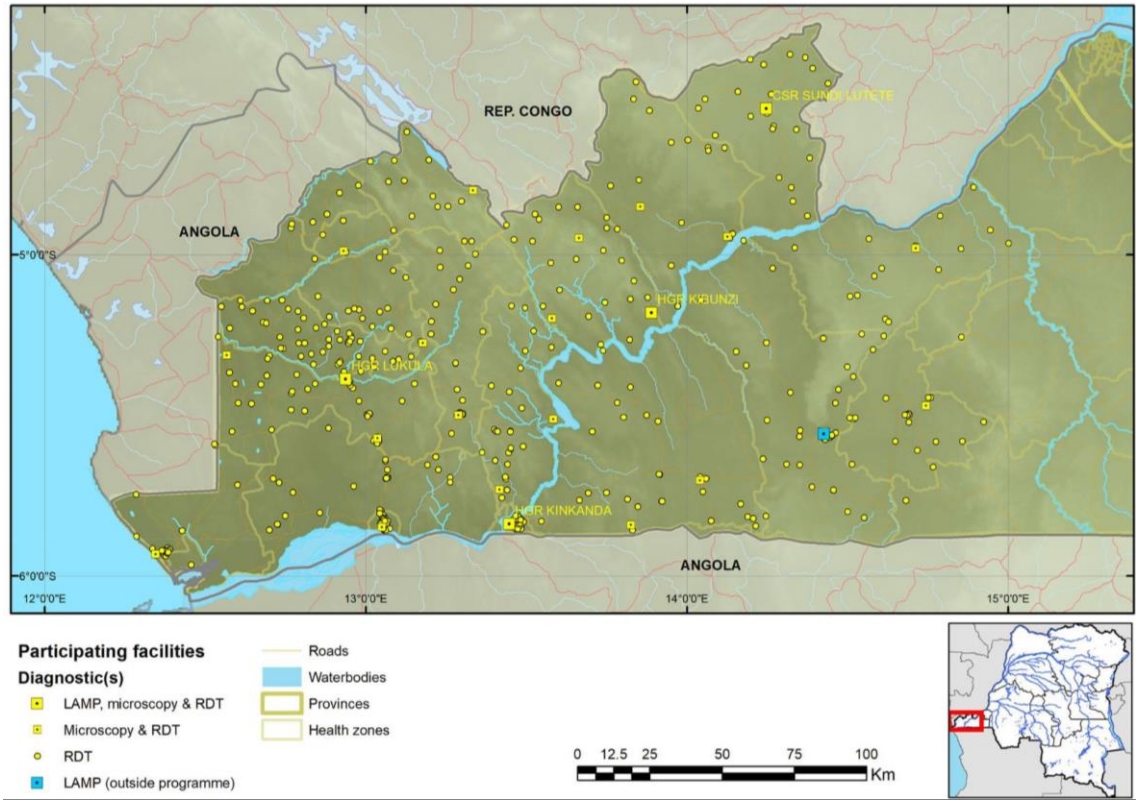
HAT cases was done in accordance with DRC national guidelines; only patients in whom trypanosomes were visualized by microscopy in body fluids were treated as HAT cases [16].

Results

Roll out of activities

Kongo Central province has 31 health zones, of which Massa, situated in the north eastern region of the province, is at the edge of the epidemiological extent of the HAT focus in the province. Among the other 30 health zones, 17 reported at least one HAT case from 2009 to 2013 (and are hereafter referred to as the HAT endemic health zones). RDTs were deployed in 620 facilities across 19 health zones (S1 Data), including the 17 HAT endemic health zones, and Nzanza and Vaku health zones, which had not reported cases from 2009 to 2013, but are surrounded by HAT endemic health zones. Among the 620 health

facilities, 23 were equipped to perform microscopy, and among these 23 health facilities, 4 were upgraded to perform LAMP. Of these 620 facilities, 603 started using RDTs and by the final quarter of 2016, 584 were still



8.2 Figure 2. Map of RDT, LED FM and LAMP sites in the Kongo Central province programme. The geodata layers were obtained from CC-BY License compatible sources: The CGIAR SRTM 90m datasets (https://bigdata.cgiar.org/srtm-90m-digital-elevation-database/). Further geodata were downloaded from Digital Chart of the World through Diva-GIS (https://www.diva-gis.org/gdata) and OpenStreetMaps (http://www.openstreetmap.org) and rendered by the authors using ESRI ArcGIS following the PLOS guidelines (https://journals.plos.org/plosone/s/figures) or were supplied by PNLTHA.

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using RDTs. These 584 facilities tested a total of 45,173 patients with HAT RDTs, and it is these 584 that were used in the analysis going forward (374 patients were screened by the 19 facilities that ceased using RDTs by the final quarter of 2016 and reporting, no cases were confirmed among these). This brought to 561 the number of RDT sites (performing only HAT RDTs), 19 LED FM sites (performing HAT RDT and microscopy) and 4 LAMP sites (performing HAT RDT, microscopy and LAMP). Enrolment of facilities was carried out gradually, starting in August 2015, with the final facilities enrolled in November 2015. All facilities except 41 RDT sites were mapped (Figs 1 & 2); for the latter, GPS coordinates were not collected by provincial and health zone teams.

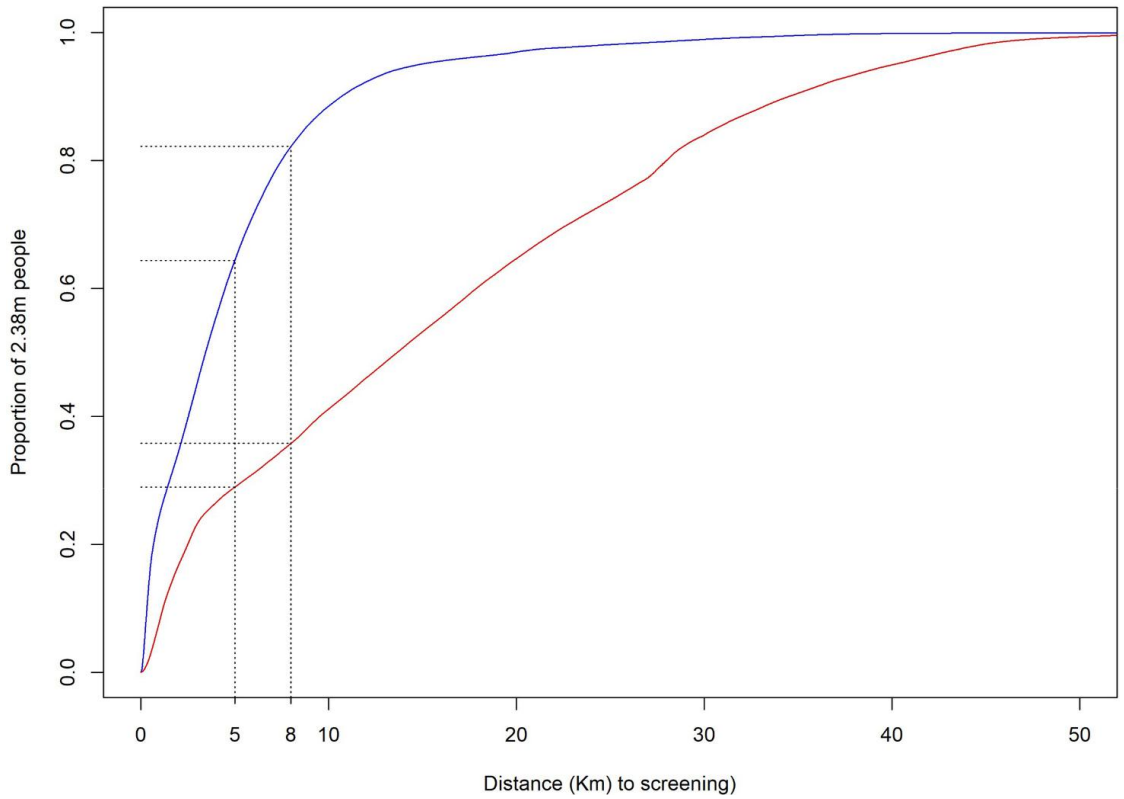
Coverage

As a result of the expansion of HAT screening activities, the median straight-line distance of the population to a facility that performed HAT RDTs is 3.4km, with 95% within 14.7km from a facility (4.9km mean distance). Comparing this to the situation when CATT was the only screening test, the median was 13.7km, with 95% of the population within 40.0km (Fig 2). The median distance for referral to microscopy is 12.0km and the corresponding mean distance 12.4km. For screening with RDTs, 64.4% of the population lives less than 5km and 82.2% less than 8km from an RDT facility, compared to screening with CATT in the past when only 28.9% lived within 5km and 35.8% within 8km from a CATT facility (Figs 3 and 4).

RDT usage

By the end of 2016, a total of 45,173 patients had been screened with RDTs, of whom 39,827 (88.2%) were tested at RDT facilities, 4,192 (9.3%) at microscopy facilities and 1,154 (2.6%) at LAMP facilities. Note that the number of RDT sites were 96.06% of all facilities. This was an average of 71.0 tests per RDT facility, (range 2–471, median = 60), 221 tests per HAT microscopy facility (range 67–815, median = 171) and 289 tests per LAMP facility (range 75–520, median = 280). The largest number of people were screened in the final months of 2016 (Fig 5). There was a lag in data reporting during the early stages of the programme when implementation was being initiated and reporting pathways were being streamlined. This initial lag in data reporting was sorted out by November 2015, hence the apparent spike in numbers screened during this month; thereafter, implementation, of the project improved with time, thanks to supporting activities such as supervision and monitoring (Fig 5). Utilization of HAT RDTs was relatively even across the province (Fig 6).

From the 45,173 patients tested with HAT RDTs by facilities that were still active at the end of the study, 925 (2.05%) were positive, 659 (71.2%) of them at RDT facilities. It is notable that whilst RDT facilities accounted for 88.2% of tests performed, they also accounted for 71.2% of positive tests.



8.3 Figure 3. Cumulative distribution plot of the population's distances to screening facilities using HAT RDTs or CATT.
The red line represents the distance to HAT screening prior to the programme, the blue line is the distance following the start of the programme and the black lines the coverage at the 5 and 8km targets of the DRC Ministry of Health. Data on population are from [19].

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Out of all the RDTs performed, 1.7% were positive in RDT facilities, 4.6% in microscopy facilities and 6.5% in LAMP facilities. Therefore, the proportion of patients that were RDT +ve among all those screened (seroprevalence) was high in microscopy / LAMP sites compared to RDT sites, a difference that was statistically significant ($p < 0.001$). However, of the 659 patients that tested positive at RDT facilities and were referred for confirmatory testing, only 263 (39.9%) completed the referral.

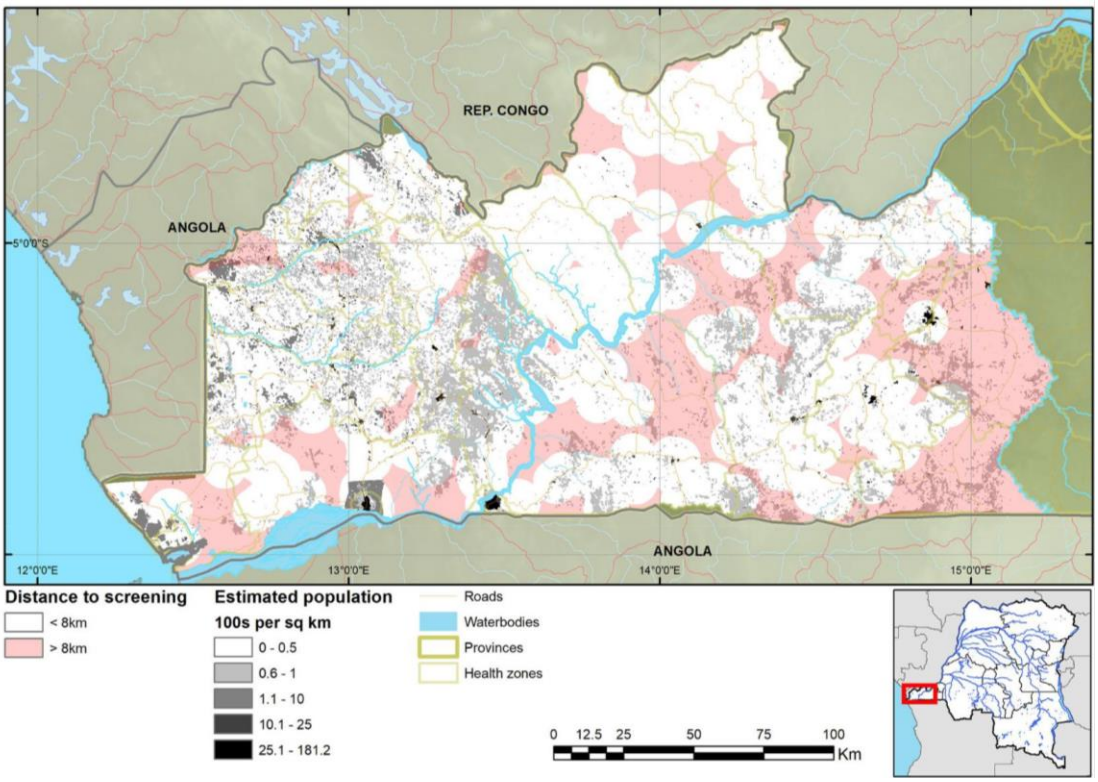
Detection of HAT cases

Out of the 296 RDT positive patients at microscopy and LAMP facilities and 263 that were successfully referred from RDT facilities, 75 were confirmed as HAT cases by microscopy during first testing, and a further 6 during a second microscopy testing after being referred with a positive LAMP test. This resulted in a total

of 81 HAT cases (14.4% of the RDT positive suspects) that were detected between November 2015 and December 2016, making an average of 69.4 cases for the year.

Eighteen (22.2%) of the cases were confirmed by LN, 3 (3.7%) by LED-FM, 32 (39.5%) by CTC, 22 (27.2%) by mAECT and 6 (7.4%) by CSF examination. From the 81 cases, 52 (64.2%) were in stage 1 of the disease (Table 2).

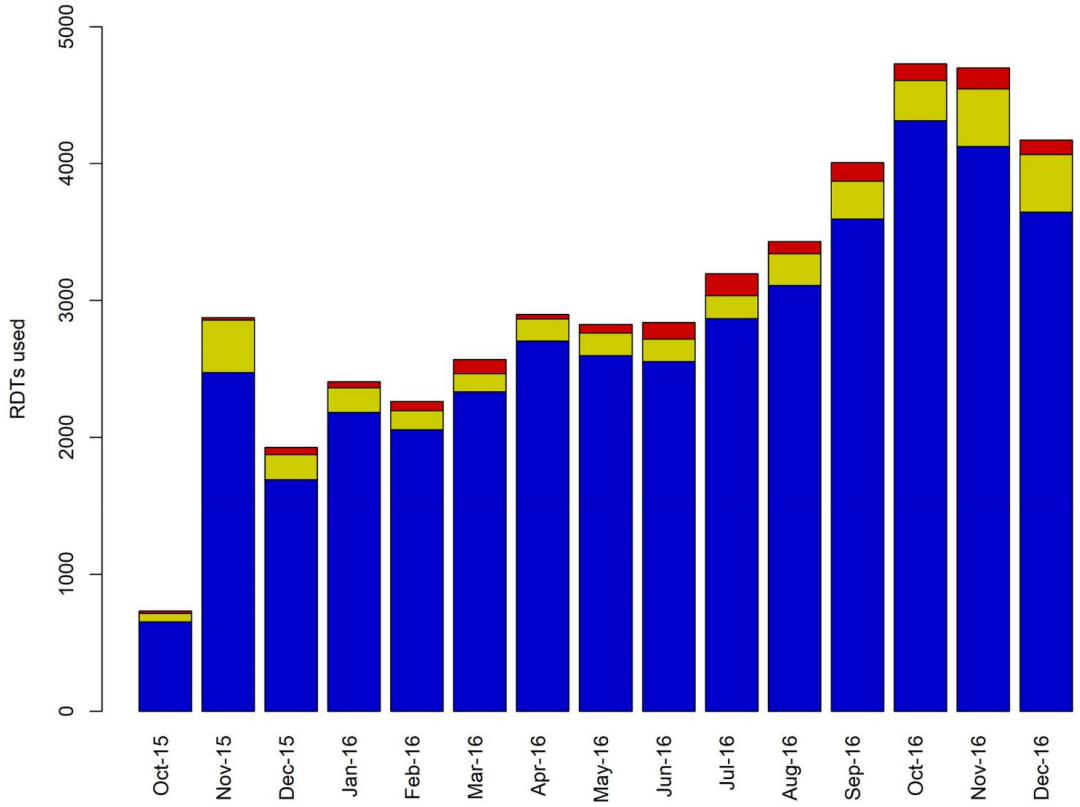
An analysis of the distance to a referral facility against whether an RDT facility screened a patient who turned out to be a HAT case shows that those RDT facilities that screened a HAT case were on average, further from a referral facility, and that those furthest from a referral facility did not screen a case (Fig 7).



8.4 Figure 4. Map of estimated population within and beyond 8km distance to health facilities screening for HAT.
The geodata layers were obtained from CC-BY License compatible sources: The CGIAR SRTM 90m datasets (https://bigdata.cgiar.org/srtm-90m-digital-elevation-database/). Further geodata were downloaded from Digital Chart of the World through Diva-GIS (https://www.diva-gis.org/gdata) and OpenStreetMaps (http://www.openstreetmap.org) and rendered by the authors using ESRI ArcGIS following the PLOS guidelines (https://journals.plos.org/plosone/s/figures) or were supplied by PNLTHA. Data on population are from [19].
https://doi.org/10.1371/journal.pntd.0008779.g004.

The HAT detection rate among all screened people was 0.098% [95% CI: 0.070–0.134] in RDT sites and 0.786% [95% CI: 0.567–1.060] in microscopy / LAMP sites, while the HAT detection rate among RDT +ve

suspects was 14.829% [95% CI: 10.762–19.709] in RDT sites (based on the RDT +ves successfully referred to microscopy / LAMP sites) and 15.789% [95% CI: 11.623–20.738] in microscopy / LAMP sites. The proportion of cases diagnosed in early stage was 71.795% [95% CI: 55.126–84.999] of cases screened in RDT sites and 59.524% [95% CI: 43.282–74.371] among the ones screened in microscopy / LAMP sites.

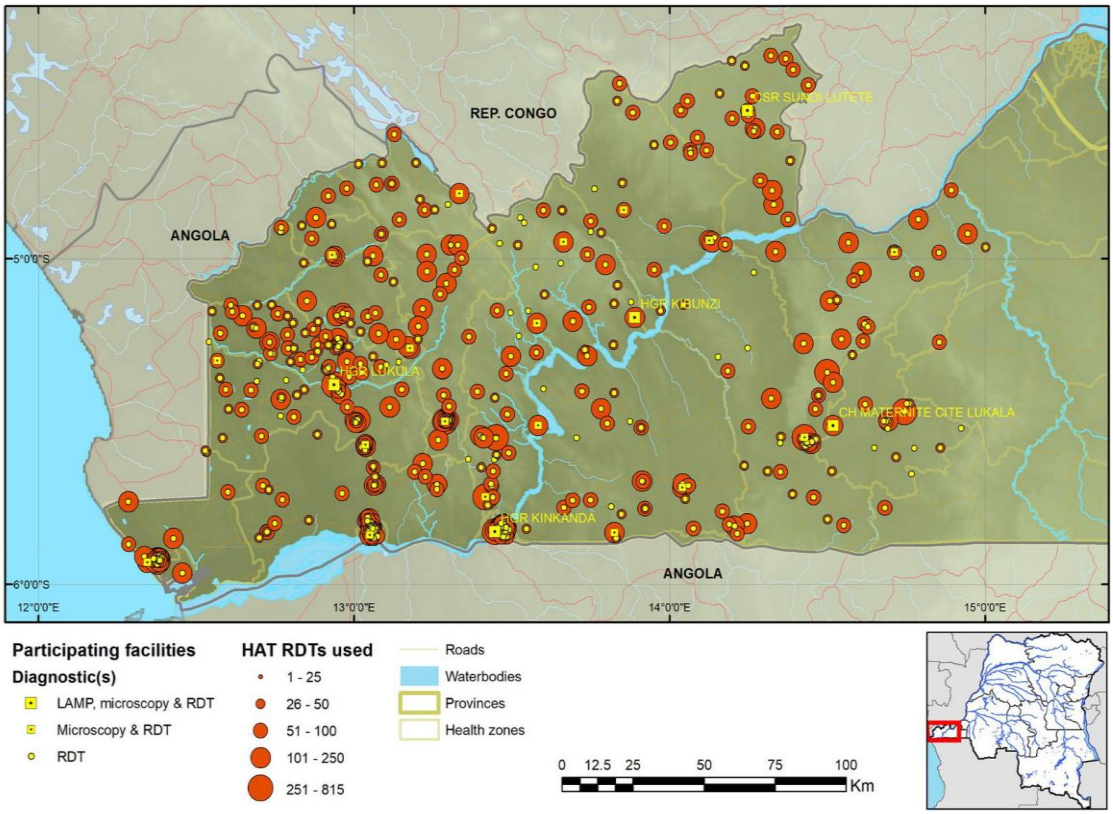


8.5 Figure 5. Number of patients screened with HAT RDTs per month. Blue represents RDT facilities, yellow microscopy facilities and red LAMP facilities
https://doi.org/10.1371/journal.pntd.0008779.g005

RDT sites, that accounted for about 95% of sites, screened about 90% of all patients. Among all patients that were RDT positive, 71% were in RDT sites. Among the RDT +ve HAT suspects referred for confirmatory testing, less than 50% (39.9%) presented at microscopy / LAMP sites, and about half (48%) of all confirmed HAT cases were first screened in RDT sites. Based on this, if a higher referral rate of RDT positive suspects had been achieved, then the proportion of diagnosed cases originating from RDT sites would have been greater than 50%.

Reactive screening

Reactive screening was carried out in 66 villages, with a total of 30,312 people screened and 55 HAT cases identified, 50 (90.9%) of whom were in stage 1.



8.6 Figure 6. Map of Kongo Central province showing the number of RDTs performed by each health facility.
The geodata layers were obtained from CC-BY License compatible sources: The CGIAR SRTM 90m datasets (https://bigdata.cgiar.org/srtm-90m-digital-elevation-database). Further geodata were downloaded from Digital Chart of the World through Diva-GIS (https://www.diva-gis.org/gdata) and OpenStreetMaps (http://www.openstreetmap.org) and rendered by the authors using ESRI ArcGIS following the PLOS guidelines (https://journals.plos.org/plosone/s/figures) or were supplied by PNLTHA.
https://doi.org/10.1371/journal.pntd.0008779.g006

During the current project the 81 HAT cases identified through passive screening came from 55 villages (the origin of 1 case was unknown and 2 were from Angola), with a range of 1 to 6 cases per village. The 39 cases screened at RDT facilities came from 31 villages located at a median of between 11.5km and 63.0km from the screening site. The 42 cases screened in microscopy / LAMP sites came from 23 villages located at a median of between 18.5km and 83km from the microscopy / LAMP screening site. Among the 55 villages that reported cases passively, 20 were subjected to reactive screening, of which 8 (40%) reported 38 cases (69.1%), with 11 cases identified in one of these villages.

Out of the 20 villages that reported cases passively, reactive screening took place in 45 villages linked to, or in the neighbourhood of the one that reported cases passively, that themselves didn't report cases passively;

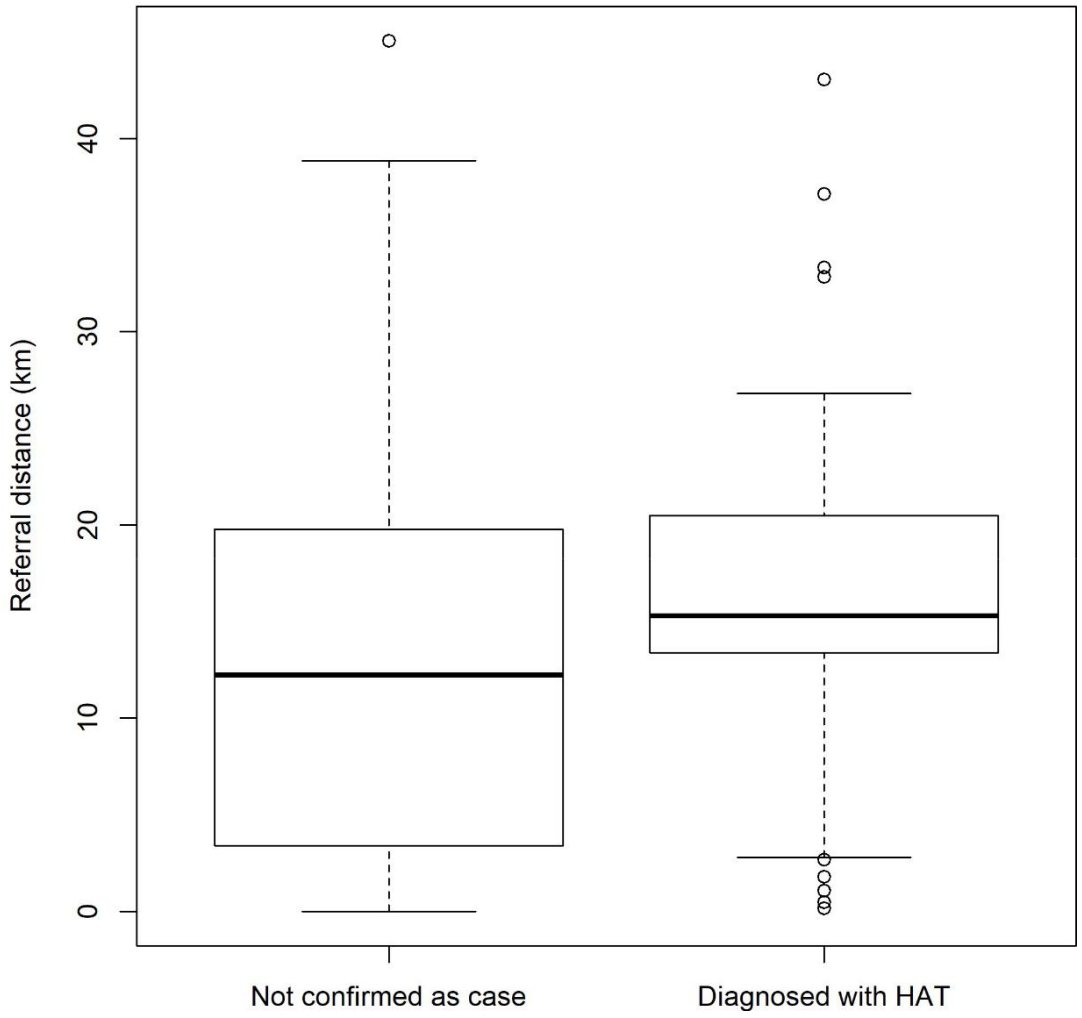
and 6 (13.3%) of these reported 16 cases (29.1%), with 8 cases in one of them. An additional case living in one of the 55 villages that reported cases passively, not subjected to reactive screening, was detected during reactive screening in a different village subjected to reactive screening.

8.2 Table 2. HAT cases identified in Kongo Central province, DRC, by stage and type of facility that screened the patients.

		HAT stage		
		Stage 1	**Stage 2**	**Total**
Screening facility	RDT	27 (33.3%)	12 (14.8%)	39 (48.1%)
	Microscopy or LAMP *	25 (30.9%)	17* (21.0%)	42 (51.9%)
	Total	52 (64.2%)	29 (35.8%)	81 (100.0%)

* one case was not staged and was therefore managed as stage 2, in accordance with national guidelines.

https://doi.org/10.1371/journal.pntd.0008779.t002



8.7 Figure 7. Weighted boxplot* of the referral distance from RDT facility against HAT confirmatory testing result
*Boxes are weighted by the number of patients screened.

https://doi.org/10.1371/journal.pntd.0008779.g007

This brought to 55 cases reported during reactive screening coming from 15 villages; 39 from 9 villages that reported cases passively and 16 cases from 6 villages that did not report cases passively, bringing to an odd ratio of villages that reported cases among the one that didn't report cases during passive screening versus the one that reported cases of 2/3 (S2 Data).

Comparison with former screening strategy

The strategy of screening for HAT (using CATT or RDT) implemented in this project would appear to be slightly superior compared to the period before the project. Assuming that the same number of patients were screened, this programme identified more HAT cases by passive screening (adjusted annual cases = 69.4) than those that were identified by passive screening from 2013 (53) to 2014 (30) (Table 3). Additionally, 64.2% of HAT cases identified in this programme were in stage 1, compared to 28.3% and 26.7% of cases by passive screening from 2013 to 2014 (Table 3).

8.3 Table 3. Comparison of the detection of HAT cases under this programme compared to the two years prior to initiation of the programme.

	2013	**2014**	**This programme–adjusted annual numbers**
	Passive screening		
Numbers screened	36229	35352	39073
Cases	53	30	69.4
Stage 1 (%)	15 (28.3%)	8 (26.7%)	44.6 (64.2%)
Stage 2 (%)	38 (71.7%)	22 (73.3%)	24.9 (35.8%)
detection rate	0.15%	0.08%	0.18%
	Active screening		
Numbers screened	51621	36626	30312
Cases	77	40	55
Stage 1 (%)	67 (87.0%)	36 (90%)	50 (90.9%)
Stage 2 (%)	10 (13.0%)	4 (10%)	5 (9.1%)
detection rate	0.15%	0.11%	0.18%

https://doi.org/10.1371/journal.pntd.0008779.t003

The HAT case numbers are adjusted because the programme was fully operational over a 14 months' period rather than one calendar year.

The total number of HAT cases detected through passive screening during this intervention was increased by 30.94% compared to 2013, and by 131.33% compared to 2014.

While the number of patients screened for HAT during this programme would appear to be equivalent to the number screened before the study, the 40 health facilities that were active prior to the current programme screened 7,073 patients (adjusted annual screened patients = 6,063) against 36,229 and 35,352 in 2013 and

2014 respectively. During this intervention, the 40 health facilities identified 44 HAT cases (adjusted annual cases = 37.7) among all the screened patients, 29 (adjusted annual number = 24.9) of them, meaning 65.9%, in 1st stage.

During the current project, active screening was conducted as reactive screening, whereby active screening is targeted at villages that reported cases passively and in their neighbourhood. We found that the detection rate during the reactive screening was higher compared to routine active screening, which targets villages that reported cases during the previous 3 to 5 years, while the proportion of cases detected in early stage was slightly higher.

Discussion

In this paper, we have described the implementation of a programme that aims to intensify screening for HAT and increase early case finding, and thus expect to contribute to elimination of HAT in Kongo Central province, a well-circumscribed and well-delimited transboundary HAT focus in DRC, Angola and Republic of Congo. This is in the context of declining HAT incidence, which requires novel means of detecting the remaining cases in a cost-effective manner [21]. By utilising the existing health facility network that has been established by the country, with the aim of ensuring that the population is within 5 or 8km at the most from a health facility [20], the existing health facilities are used to improve population coverage.

In the DRC, Kongo Central contributes a relatively small number of cases to the national total. The results obtained in this programme can be used as an example of a strategy leading to HAT elimination, that can be implemented at a national scale. Furthermore, the Kongo Central province accounts for the majority of HAT cases in the transboundary focus of Angola, Republic of Congo and the DRC [22, 23], and therefore, driving down case numbers in this region could reduce the overall case numbers, and risk of reintroduction throughout the region.

Similar passive surveillance systems that have been implemented in Angola and Republic of Congo will complement these elimination efforts. However, to envisage extending such programme to national scale, other aspects need to be considered, including the cost and feasibility.

One output that suggests that this intervention could be contributing in driving elimination is that majority of the HAT cases that were identified were in stage 1, regardless of whether they were screened at RDT or microscopy/LED facilities, meaning that they were being identified earlier in the course of infection, reducing their potential of contributing to disease transmission by acting as reservoirs. This is important in limiting disease spread (transmission) in the community. The rate at which cases present in stage 1 increased by around 40%. This suggests that people are presenting earlier in the course of infection because there is easier access to diagnostics at the local health facilities, rather than waiting until symptoms are distinctive of HAT before

travelling long distances to a specialist diagnostic facility. This also means that patients' suffering is reduced, and that they can be treated with greater safety than if they are diagnosed during stage 2. It would appear that the number of patients that were screened in the passive setting remained relatively constant when compared to previous strategies, while the number of cases identified increased. In fact, the patients screened with RDTs were systematically the clinical suspects compared to previous strategies where CATT was used as screening test. The total number of people screened in the health facilities that were active prior to the intervention was at least five times higher before the intervention compared to the number screened during the intervention. In fact, through this strategy the clinical screening was the 1st screening step prior to RDT screening and therefore, the level of HAT suspicion in those tested with RDTs was higher than in previous strategies.

A core component of this strategy is the reactive screening in villages from which HAT cases were identified by passive screening or in the neighbourhoods. In regions such as Kongo Central that have a lower prevalence than other provinces of DRC, this reactive screening approach may be a good way of identifying local outbreaks. Hence, in this study, among the villages that identified cases by passive screening, 20 were followed up with reactive screening and cases identified in 8, with 11 cases in the worst affected village. Reactive screening also identified cases in 6 of 45 villages linked to or in the neighbourhood of villages where cases had been detected passively.

The detection case rate was high in this project compared to routine active screening in previous years. This may be because reactive screening as implemented in current project allows better targeting of endemic villages, but needs to be supported by a wide coverage and efficient passive screening strategy. Such passive screening would minimize the number of endemic villages that are missed by reactive screening.

The observed high rates of case detection in passive screening were in spite of the relatively poor referral rates of RDT positive suspects from RDT facilities, which was 41.2%. While it cannot be expected that this means that 58.8% of cases from RDT facilities were missed, further research would be required, to estimate the proportion of those that did not complete the referral that could have been true cases. It can however be assumed that if patients continued feeling unwell after being referred, then they would complete the referral, and that majority of patients that are not confirmed because of not completing the referral would have a self-limiting illness and will recover. However, among the 58.5% that do not present will be some that are HAT cases and did not refer due to lack or resources, time, or other reasons, or possibly died prior to completing referral. In this study, HAT detection rate among clinically suspected patients was statistically significantly higher in microscopy / LAMP sites compared to RDT sites, while there was no statistically significant difference with regard to the proportion of HAT cases among the RDT +ve suspects. The low HAT detection rate among all those screened in RDT sites may be a reflection of easy access (economic and geographic), and the fact that any patient presenting with even a few clinical signs that could be suspected of HAT are tested

with the RDT (which could result in low specificity of the clinical suspicion step) compared to specialized HAT confirmatory sites. The fact that the proportion of confirmed HAT patients among all persons tested with RDT were statistically significantly higher in RDT sites than in microscopy / LAMP sites could be a reflection of an early clinical suspicion in RDT sites compared to microscopy / LAMP specialized sites. The sensitivity of the LED FM diagnostic test was low in the current intervention compared to previous findings, but did not have an impact on the performance of the whole confirmatory algorithm, as the most sensitive tests were included [6, 7]. Additional strategies, such as mobile teams that track down RDT positive suspects and perform confirmatory testing, coupled with intensified community sensitization, could improve these referral rates. Nkieri et al (2020) suggested an active follow-up implemented by Health Zone teams, using community health workers to locate suspects to be followed up [24]. The cost-effectiveness and feasibility of those strategies in relation to current strategies towards the HAT elimination goal have to be evaluated and considered.

Limitations

This study had a number of limitations, that would need to be addressed in case such an intervention is implemented in other settings. The cost and the cost-effectiveness of this intervention to contribute to the HAT elimination objective was not evaluated. This could vary greatly depending on the accessibility in a region. The referral rate could also influence the effectiveness of such an intervention. The proportion of suspects that did not present themselves for confirmatory parasitological testing could have compromised the results. Strategies to reduce this proportion as much as possible towards 0% must be built and evaluated.

Conclusion

The analysis presented here has demonstrated that this strategy is successful in increasing early HAT case detection, particularly in a large area (38,900km^2) that has a relatively sparse distribution of HAT cases. Further work should be conducted to determine the costs of the strategy, including whether it could be one of the cost-effective strategies contributing to HAT elimination. In the next phases of this programme, the number of health facilities screening for HAT will be scaled back to evaluate whether a similar level of success can be achieved whilst employing a smaller number of health facilities.

Supporting information

S1 Data. Health facilities in Kongo Central province prior and during the project.

(XLSX)

S2 Data. Spread of HAT cases in Kongo Central province by passive and reactive screening.

(XLSX)

S1 Text. Health Facilities Mapping Survey Data Collection Form.

(DOCX)

S1 Fig. The *T. b. gambiense* human African trypanosomiasis (gHAT) diagnostic workflow implemented in Kongo Central province. Gland puncture (GP); Whole Blood (WB); Acridine Orange-Fluorescence Microscopy (AO-FM); Capillary Tube Centrifugation (CTC or

mHCT); mini-Anion Exchange Centrifugation technique (mAECT); buffy-coat (bc).

(TIF)

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Writing – review & editing: Crispin Lumbala, Simon Kayembe, Jacquies Makabuza, Pascal Lutumba, Jean-Pierre Van Geertruyden, Paul R. Bessell, Joseph Mathu Ndung'u.

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Chapter 4

General discussion and conclusion

HAT epidemiological trend and elimination objective

We found that *gHAT* prevalence in DRC decreased substantially. However, the analysis at subnational level revealed that some provinces remained at an important level of gHAT. The provinces of Maindombe and Kwilu remained the most prevalent followed by Kasai Oriental and Kongo Central. The provinces of Nord Ubangi and Sud Ubangi experienced a stronger decrease and were among the least prevalent provinces in 2016. Maindombe and Kwilu provinces, accounted for 22.3% (25 out of 112) of all health districts (HD) at moderate, high or very high gHAT transmission intensity for the period 2000-2004, and 53.7% (22 out of 41) for the period 2012- 2016. Nord Ubangi and Sud Ubangi accounted for 17.9% (20 out of 112) of all HD with moderate, high and very high transmission intensity for the period 2000-2004 and 2.4% (1 out of 41) for the period 2012-2016. Kasai Oriental province evolved from 18.8% (21 out of 112) to 14.6% (6 out of 41), Kongo Central from 9.8% (11 out 112) to 7.3% (3 out of 41) of HDs at moderate, high and very high transmission intensity between the periods 2000-2004 and 2012-2016 respectively. This trend in disease at subnational level was confirmed by the analysis of HAT transmission intensity by several authors (Franco *et al* (2020) (1). The trend of disease between provinces from 2000 to 2016 confirmed what was observed by several authors (Lutumba *et al* (2), Lumbala *et al* (3) and Davis *et al* (4)) i.c. that the decline at national level was mainly attributable to Nord Ubangi and Sud Ubangi provinces, while the situation was stationary in Kongo Central, Kasai Oriental, Sankuru, Kasai, Kasai Central, Maindombe, Kwilu and Kwango provinces. Based on the above, while HAT control in Nord and Sud Ubangi provinces appears to be on track, the disease has proven more difficult to tackle in other provinces, such as Kwilu, Maindombe, Kasai and Kongo Central province. Factors like HAT screening coverage, population attendance rate to active screening, the proportion of patients who received treatment, and the therapeutic efficacy rate have been identified as predictors to disease trend in different zones (2, 5). Nord and Sud Ubangi provinces had the highest covered rate compared to others (2) and very high attendance rates while attendance rate was low and therapeutic failure rate was high in Kasai oriental province (6).

The analysis at HD level showed that 16.0% of the 257 endemic HDs had not yet reached the target set against the main indicator of HAT elimination as PHP according to Franco *et al* (2020) in 2016 (as having reported an average of ≥1 case/10,000 people/year over the 5-year period from 2012 to 2016). At country level, across all 257 HDs in 2020, the reported 1.8 cases/100,000 persons/year shows that the goal of eliminating HAT as PHP was achieved considering the threshold. However, apart from the fact that this needs to be verified at the level of each HD, HAT control and surveillance activities need to be assessed. Insufficient active screening

and underreporting may present transmission as much lower than it actually is (4). For this reason, qualitative assurance indicators of control and surveillance activities were defined as secondary and complementary indicators relating to active and passive case-finding, case management and vector control (1).

Although the number of people actively screened annually remained almost stable, active screening was poorly implemented according to the recommended algorithm (7). Indeed, the average proportion of villages targeted for annual active screening for 3 consecutive years that were actually visited by a mobile team between 2012 to 2016 varied from 12 to 20% in 5 sampled provinces (Maindombe, Kwilu, Kasai Oriental, Kongo Central and Sud Ubangi). Although few villages were actively screened according to the recommended algorithm, the magnitude of the disease decreased substantially and sustainably (70% decrease in HAT cases from 2012 to 2016). A more in depth analysis reveals that while less than 20% of villages were actively screened as recommended, at least 60% of these targeted villages were visited at least once during the period considered (2012-2016). We may conclude that visiting an eligible village at least once during the recommended 3 consecutive years contributes substantially to HAT control even if the proportion of villages visited per year is low. This point is in fact corroborated by Davis *et al* (2021) who reported that the cost-effectiveness of AS (costs and DALYs averted) were very similar when cessed after one, two, or three years of zero cases. However, he adds that cessation after three years of zero cases is appropriate, likely more cost-effective, and recommended for a moderate-to-high endemicity HD in the DRC—in line with current WHO guidance (4). Also, Castaño *et al* (2020) reported that two or three consecutive years without detected cases provided greater confidence of reaching the end of transmission (EOT) than a single year (8).

Our data also showed that adherence to the algorithm is associated with the identification of more HAT cases in AS eligible villages visited by a mobile team compared to villages not eligible to AS according to the algorithm but visited by a mobile team. Indeed, the proportion of AS eligible villages that reported cases was at least 5 times higher than the proportion of non-eligible villages that were actively screened. Therefore, beyond the fact that it is not efficient to visit a village that is not eligible to AS, not visiting an eligible village would mean that a case of HAT would have been missed and would remain as a source of contamination for the community.

In view of the above, we can conclude that while AS is the main screening strategy supporting HAT control in the DRC (5), the way it has been implemented does not provide sufficient confidence that it will lead to sustainable progress towards elimination of the disease.

Regarding passive surveillance, it was reported that it was increasing year by year with more health facilities providing HAT diagnosis and treatment and thus increasing the coverage of the population at risk (1, 9, 10) . However, our analysis revealed that in 2017, nearly two-thirds of endemic health districts were not covered by a health facility implementing the full range of HAT activities and approximately 40% of endemic health areas

were not covered by a health facility capable of screening for HAT. Given the sharp decline in the number of HAT cases in DRC, passive screening and integration of activities into PHC polyvalent health facilities is the promising strategy to ensure the post-elimination surveillance required for sustainable elimination to an EOT (11). Therefore, to ensure surveillance of HAT in the post-elimination period, passive screening coverage must be improved.

In view of all the above, we may conclude that elimination of HAT as PHP is truly within reach, but observed progress may be overestimated and thus the extent of the disease underestimated. Indeed, the underperformance of active screening and the low coverage of passive screening may miss cases that could be a source of resurgence in the future. These do not appear to be sufficient to ensure surveillance in the post-elimination period and to provide sufficient confidence in sustainability towards an EOT. The relative increase of number of cases noted in the country between 2020 and 2021 underscores this concern (PNLTHA annual reports).

Therefore it is crucial to confirm the observed HAT trend in DRC, which appears to be almost eliminated based on current results, and to improve coverage and implementation of active and passive screening strategies countrywide to ensure HAT elimination as PHP. To contribute, we focused our research on innovative diagnostic tools and strategies.

Innovative new diagnostic tools and strategies and their contribution to elimination of HAT

The detection and control of HAT has long relied on active screening by on-truck mobile teams as the primary control strategy with CATT as the used screening test. Given the current low endemicity, active screening has become less cost-effective than passive screening. Unfortunately, the integration of activities in PHC settings encountered a number of issues due to the requirements of CATT as a screening test and the complexity of parasitological testing requiring specialized personnel (7, 12). In accordance with WHO guidelines and recommendations, we developed and successfully tested; the first ever thermostable HAT screening test, requiring no electricity or cold chain, provided in a single format and easy to use, the SD BIOLINE HAT RDT ® based on native trypanosome antigens (1st generation HAT RDT). To overcome the weaknesses of this native antigen-based HAT RDT (risk of accidental contamination during laboratory manipulations, risk of stock-out in case of lack of native antigens, cost), we extended our work, contributing to the development of a SD BIOLINE 2 ® HAT RDT based on recombinant antigens (2nd generation HAT RDT). The latter proved to be as effective as the 1st generation HAT RDT (13-15).

Diagnostics' algorithms based on SD BIOLINE ® HAT RDT were more cost-effective and ease to be implemented in passive screening by fixed health facilities while it was less cost-effective when used during active screening by on-truck mobile teams with 7-9 members team. However, as alternative to current active

screening by on truck 7-9 persons mobile team, a novel active screening based on light mobile teams made of 2 team members traveling by motorbike and using HAT RDT instead of CATT(16) should be explored.

Given the ease of use and integration of HAT in PHC settings, we built and tested a strategy based on HAT RDT in one of the DRC provinces, integrating also malaria RDT into the HAT diagnostic process. Every patient tested negative to malaria RDT was subjected to HAT RDT. The HAT RDT positive patients were then submitted to confirmatory testing or referred for confirmatory testing. Unconfirmed patients were subjected to LAMP test, a molecular test, as 2nd level of screening at health facilities equipped to perform LAMP or their blood sample was dried on filter paper and sent for testing at LAMP sites, as LAMP testing was performed at limited referral centers. This strategy was combined with reactive screening i.c. the village of origin of a confirmed HAT case during passive screening was subjected to active screening up to three months following the diagnosis . This strategy proved to be effective in detecting a high proportion of cases at an early stage, breaking the paradigm that passive screening does not allow for early case detection, which is in fact essential to stop disease transmission. However, this strategy encountered some limitations including the fact that successful referral of HAT RDT positive individuals to microscopy sites for confirmatory testing remained the main issue to be addressed. The low referral rate in such a strategy was also reported by Snijders R. *et al*, where only 20% of HAT RDT positive patients completed the recommended referral. As 72% of the referred suspects were confirmed HAT cases (17), the low referral rate needs to be addressed urgently.

Another concern regarding integration of HAT in polyvalent health facilities in contrast to HAT-specialized health facilities is the lack of commitment especially as the disease is becoming rare. Therefore, it has been suggested to integrate HAT diagnostic and management activities into the management process of other similar tropical diseases, such as malaria. We tested a prototype of a combined HAT/malaria RDT. This HAT/malaria RDT, more than a simple test, is an integrated diagnostic strategy manner for malaria and HAT.A blood sample collected from patients who are tested positive for the HAT/malaria RDT HAT band will be equally submitted to HAT confirmatory testing or sent to a referral health center for confirmatory testing.

In summary, HAT RDT is an alternative to CATT increasing coverage of passive HAT screening. Integration HAT RDT into an algorithm and strategy, integrated with other tropical disease management would allow to reduce medical delay, thus earlier diagnosis and better integration of HAT control in PHC polyvalent health facilities.

Given the current trend of HAT prevalence toward elimination, passive screening, complemented by less expensive (re-)active screening approaches, can maintain the trend toward HAT elimination as PHP and post-elimination surveillance. Our work resulted in the development of various individual, thermostable and easy-to use screening tests. However, these tests may face some operational limitations, mainly due to the specificity with very low HAT prevalence resulting in low PPV. This will lead to few confirmed HAT cases among a

large number of positive HAT RDT. This could decrease the confidence in positive HAT RDT results, reluctance to perform confirmatory testing when few or no clinical signs and symptoms are present, which is common in gHAT, with a potential risk that true cases are missed. To improve specificity and thus PPV, we may combine different HAT RDTs through a given algorithm or combine different HAT RDT with other serological or molecular screening tests.

Limitations of studies

Mobile team visiting endemic villages could not distinguish if participants were from the village actually visited or from another one endemic village in neighbourhood. This could underestimate the proportion of endemic villages covered by mobile team through active screening. The feasibility and acceptance of HAT RDT in active screening has also not been evaluated. Aside SD BIOLINE HAT RDT®, 3 other RDTs have been developed and our studies didn't evaluate the feasibility of HAT active screening with HAT RDT and its comparison with CATT on truck mobile teams which have larger volume.

Threats and unaddressed areas towards HAT elimination

HAT/ malaria RDT was evaluated as a prototype and showed encouraging results. Performance evaluation of a final product and its feasibility, acceptance and contribution to HAT surveillance in field conditions is an important step to cover. As HAT screening via test HAT/malaria RDT needs a confirmatory step in case of HAT positivity, while its diagnostic test for malaria, it is important to explore an efficient algorithm and strategy for a successful integration in polyvalent health facilities.

Another concern still to be addressed is the retrieval rate of patients having a confirmed HAT test at a referral centre. This will need to be further explored how to improve this retrieval rate.

Conclusions and perspectives

To sustain current results towards HAT elimination / transmission interruption, the integration of HAT control and surveillance into the primary health care (PHC) system must be reinforced and closely monitored and evaluated. Active case-finding activities must be maintained where necessary and/or reactive screening established, in complement to the passive case detection, especially in those areas where the risk of infection remains high and where resurgence may occur.

This project contributed to integrate HAT activities into the PHC system by providing single format thermostable rapid diagnostic tests (RDT), easy to use to any polyvalent health worker (13-15). However, the lower specificity of current HAT RDTs remain an issue in low endemic settings, as resulting in high number of false positive individuals (13, 15, 18, 19). Further explorations are needed to build more cost-effective algorithms, accessible to polyvalent health workers. Combining different RDTs and other potential tests with

or without CRS procedures, which are challenging for polyvalent health workers, could be explored (7, 20, 21).

Through this research study project we demonstrated that it was possible to detect HAT case at early stage in passive screening strategy with an increased screening coverage. To overcome limitation to a successful and effective passive case detection in polyvalent fixe health facilities, especially in HAT low prevalence settings we developed a HAT/malaria combine RDT. More than being just a single RDT, it represents a strategy where HAT screening is integrated into diagnosis of malaria with which many similarities exist (22), really useful to maintain HAT surveillance in low and very low prevalence situations, as in HAT post-elimination settings. Research in field conditions are crucial to confirm good performance found for the prototype HAT/malaria combined RDT.

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