Evaluation of the Re-emergence Risk of Human African Trypanosomiasis in the Southwestern Burkina Faso, A Gold-Bearing Mutation Area

Evaluation of the re-emergence risk of human African trypanosomiasis in the southwestern Burkina Faso, a gold-bearing mutation area

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Abstract

Purpose: The boom in Burkina Faso's artisanal gold mining since 2007 has attracted populations from Côte d'Ivoire and Guinea, which are the West African countries most affected by human African trypanosomiasis (HAT) and therefore increases its risk of re-emergence. Our aim was to update the HAT data in Burkina Faso in the risk of the re-emergence context with the advent of artisanal gold mining.

Methods: The study was carried out in the southwestern Burkina Faso where entomological surveys were conducted using biconical traps in March 2017. Follow by an active medical survey in April 2017, which was targeted the gold panners in 7 villages closer to artisanal gold sites, using CATT, mini-anion exchange centrifugation technique, trypanolysis test (TL) and ELISA test to measure human/tsetse contacts. The buffy coat technique and the TL were also applied in pigs to check their reservoir role of human trypanosomes.

Results: Our results have shown no case of HAT among 958 individuals tested and all the 50 pigs were also negative, but the level of antibodies against tsetse saliva evidenced by ELISA revealed low human / tsetse contact. Moreover, gold panners practise agriculture and breeding in an infected tsetse area, which are increased the risk.

Conclusion: Our results illustrate that the risk of re-emergence is low. The passive surveillance system implemented in 2015 in southwestern Burkina Faso is needed to increase the sentinel sites to better cover this area by taking into account the gold mining. Finally, awareness-raising activities are needed among populations about HAT.

Keywords: Human African trypanosomiasis, risk of re-emergence, artisanal gold mining, passive surveillance, Burkina Faso.

Introduction

Trypanosomes are flagellated parasites, transmitted by the tsetse fly, that cause human African trypanosomiasis (HAT) or sleeping sickness [1]. *Trypanosoma* (*T.*) *brucei* (*b.*) *gambiense* is responsible of a chronic form of the disease in West and Central Africa with 98% of the reported cases. The World Health Organization (WHO) has reported 2 184 cases in 2016 and 977 cases in 2018, and aims to achieve interruption of transmission (zero cases) by 2030 [2].

Since the 1970s, the HAT prevalence has decreased considerably in Burkina Faso due to vector control and medical surveys, high population growth and climate change [3]. As a result, the last native case of HAT of the 1930-1980 epidemic period was diagnosed in 1993 in Bobo-Dioulasso [4]. Surprisingly, a new native HAT case was recently diagnosed in 2015 in Banfora [5]. Generally, few imported cases from Côte d'Ivoire were detected in Burkina Faso [3, 6] due to the significant migratory movements of populations between these two bordering countries and their important historical, social and economic connections [7]. However, an animal origin could also be suspected due to the massive cattle transhumance between the Ivorian foci and the Banfora area that is still tsetse infested [5]. This last case confirmed that there is still a risk of HAT re-emergence in areas where the vector persists in Burkina Faso, mainly in the southwestern part of the country [3, 8, 9]. This justified the implementation of HAT passive surveillance in this area [5].

In the context of the current HAT elimination initiative, WHO supports low endemic countries such as Benin, Mali, Togo and Burkina Faso, for the establishment of a passive surveillance system based on sentinel sites in the main health referral structures in areas at risk [10]. Six sentinel sites were established in April 2015 in Burkina Faso. These sites are located in three regions (Hauts-Bassins, Cascades, Sud-Ouest) in the southwestern part of the country, including a peripheral health structure and a hospital per region. In the sentinel sites, HAT

clinical suspects are tested using a Rapid Diagnostic Test (RDT). Next, a RDT-positive subject is tested using immune trypanolysis (TL) and parasitological tests for TL-positive subjects at the Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES, Bobo-Dioulasso, Burkina Faso). The implementation of this passive surveillance allowed the detection of the native case of HAT in 2015 [5].

The boom in Burkina Faso's gold mining in 2007 [11], has caused the proliferation of artisanal gold sites [12]. Indeed, the results of the national survey of the gold panning sector in 2016, revealed that artisanal gold mining employed 140 196 workers in 448 functional mining sites. These sites are located in 12 of the 13 regions of Burkina Faso including the southwestern region that was leading in artisanal gold mining in 2016 [13]. Located in gallery forests or along watercourses infested by tsetse flies [8], these gold sites probably facilitate human-tsetse contact. Furthermore, they attract populations from neighboring countries [12], in particular from Côte d'Ivoire and Guinea, the most HAT-affected countries in West Africa [6, 10].

The objective of this study was to evaluate the re-emergence risk of HAT in southwestern Burkina Faso in this context of proliferation of gold panning in tsetse-infested areas, through epidemiological (human, animal and entomological surveys) and geographical investigations. The expected outcomes are to propose adapted surveillance strategies for HAT control.

Materials and Methods

Study area and sampling sites

The study area is the Comoé watershed in the southwestern part of Burkina Faso, located between 9°35' and 11°05' North latitude and 3°30' and 5°30' West longitude. This watershed covers an area of 17 000 km^2, and is composed of two hydrographic units each comprising a permanent stream: the Comoé to the East and the Léraba to the West of the basin (Fig. 1). Functional gold mining sites (n = 42) have been inventoried in 38 villages during preliminary investigations based on field observations, questionnaires, informal interviews and information collected in the health information and monitoring and evaluation centers database. The number of gold sites were in 2017: 22 in Cascades, 3 in Hauts-Bassins and 17 in Sud-Ouest regions. We selected 11 villages closer to gold mining sites, located within a radius of 5-10 km from the main and secondary rivers in the Comoé watershed and belonging to three medical districts which are Banfora sanitary district (Cascades), Dô district (Hauts-Bassins) and Kampti district (Sud-Ouest).

Entomological and geographical surveys

Entomological surveys were conducted in these selected villages using biconical traps at fixed sites for 48 hours in March 2017. The deployment was made in the most favourable sites to tsetse populations (vegetation around villages, watering points and agricultural sites). Each trap was geo-referenced using a Global Positioning System and cages were harvested. Trapped flies were counted and apparent density of tsetse flies per trap per day (ADT) was calculated [14].

We collected also information regarding activities at risk for HAT transmission such as source of water supply, agriculture, presence of domestic animals (cattle, pig, sheep, goat) potentially reservoirs of *T. b. gambiense* [16], attendance by migrants and census of functional

111 gold sites. These data were recorded during preliminary investigations in the 11 selected
112 villages.

113 The midgut, the proboscis and the salivary glands of alive tsetse flies caught were
114 dissected in order to look for possible trypanosome infection using microscopy [15]. The organs
115 were preserved at -20°C for ulterior molecular diagnosis by polymerase chain reaction (PCR).

116

117 **Epidemiological investigations**

118 ***Human medical survey***

119 The serological test Card Agglutination Test for Trypanosomiasis (CATT / *T. b. gambiense*)
120 [17] was first performed from blood taken by finger prick particularly targeting gold panners in
121 April 2017. The positive individuals in CATT/blood were then tested in CATT using two-fold
122 plasma dilutions (CATT/plasma). For each serological suspect (CATT/plasma end titer ≥ 1/4),
123 the mini-anion exchange centrifugation technique (mAECT) was performed on buffy coat (BC)
124 [18]. For the CATT/plasma positive individuals, the PCR was also performed using blood
125 samples.

126 For participants older than 5 years, an additional heparinised capillary tube was sampled
127 to collect plasma immediately frozen at -20˚C in the field and at -80˚C in the laboratory for
128 subsequent trypanolysis (TL) and ELISA tests (see below).

129

130 ***Animal survey***

131 Pigs that are known to be potential reservoirs of *T. b. gambiense* [19] were investigated when
132 present during the medical survey. For each animal, 5 ml of blood were taken from the jugular
133 vein and were used to perform parasitological diagnosis in the field by the buffy coat technique
134 (BCT) [20]. In addition, 1 ml of plasma and 1 ml of blood were aliquoted for TL and PCR,
135 respectively.

136 ***Trypanolysis test***

137 Trypanolysis test was performed on all human and pig plasma samples. Because of a limited
138 volume of plasma, we used only one cloned population of *T. b. gambiense* variant antigen type
139 LiTat 1.3 as previously described [21, 22]. Briefly, 25 µl of plasma sample were mixed with 25
140 µl of guinea pig serum, to which 50 µl of a suspension with 10^7 trypanosomes/ml prepared from
141 infected mouse blood were added. After 90 min of incubation at room temperature, the
142 suspension was examined directly by microscopy (x400). Sample was considered positive
143 (TL+) when more than 50% of the trypanosomes were lysed.

144

145 ***Evaluation of anti-saliva responses in ELISA test***

146 IgG antibodies (Ab) against specific salivary tsetse peptide $Tsgf1_{18\text{-}48}$, were determined by the
147 indirect-ELISA technique according to Dama et al. [23] with minor modifications. The
148 modification was the colorimetric development that was carried out using K-Blue TMB
149 substrate (Neogen Corp, Lexington, KY). Optical density (OD) was measured at 620 nm. Each
150 plasma sample was analysed in duplicate in antigen wells and, in parallel, in a blank well
151 containing no peptide solution (OD_n) to control non-specific reactions between the plasma and
152 the reagents. The individual results were calculated in ΔOD measured according to the
153 following formula: $\Delta OD = OD_x – OD_n$ where OD_x is the average of the duplicate wells and OD_n
154 the optical density of the well without peptide.

155

156 ***PCR diagnostic***

157 DNA extraction was performed using the DNeasy Blood and Tissue kit (Qiagen) following the
158 manufacturer's instructions. A PCR using the highly sensitive TBR1/2 primers (TBR1: 5-CGA-
159 ATG-AAT-ATT-AAA-CAA-TGC-GCA-G-3; TBR2: 5-AGA-ACC-ATT-TAT-TAG-CTT-

160 TGT-TGC-3), which target a multicopy sequence specific to *Trypanozoon* [24] was performed
161 with the DNA samples [25].
162 PCR was carried out in a final volume of 25 µl containing 10 pmol of each primer, 0.2
163 mM of each desoxyribonucleotide, 1X incubation buffer with 1.5 mM $MgCl_2$, 0.5 units of Taq
164 polymerase and 2.5 µl DNA sample. PCR products were migrated on a 2% agarose gel. When
165 the expected 177 bp product was visible, the PCR was considered positive.
166
167 **Statistical analysis**
168 The IgG Ab responses were analysed with R 4.0.3 [27]. After verifying that ΔOD values did
169 not assume Gaussian distribution using Shapiro-Wilk normality test, the non-parametric
170 Kruskal-Wallis test was used for comparison of Ab levels between the different villages closer
171 to gold panning sites. Values of $p < 0.05$ were considered significant.

Results

Entomological and geographical investigations

A total of 60 tsetse flies were collected in 51 traps: 20 traps deployed in villages of Banfora sanitary district (Cascades region), 19 in Dô district (Hauts-Bassins region) and 12 in Kampti district (Sud-Ouest region). The tsetse flies captured were 3 at Banfora, 53 at Dô and 4 at Kampti districts (Table 1). Two species were caught including *Glossina* (*G.*) *palpalis gambiensis* at 90% (54/60) and *G. tachinoides* at 10% (6/60). An overall tsetse infection rate was 30% (18/60).

The table 2 shows the information in 11 villages subject to preliminary investigations. The considered epidemiological criteria were source of water supply, activities at risk for HAT transmission, presence of domestic animals potentially reservoirs of trypanosomes, attendance by migrants and census of functional gold sites. Finally, we selected 7 villages among 11 previously identified for epidemiological investigations. These sites were selected in relation to a watercourse with a distance of 5-10 km, the ADT of tsetse flies and the presence of people coming from Côte d'Ivoire or Guinea.

Parasitological, serological and molecular diagnosis

Seven villages closer to gold mining sites have taken part to the medical survey: 3 villages in Banfora sanitary district, 3 villages in Dô district and 1 village in Kampti district. Out of the 958 individuals tested, both CATT/blood and CATT/plasma were positive for 5 persons (Table 3). These CATT-positive individuals were mAECT-negative. All subjects were also TL-negative. In addition, a total of 50 pigs were tested but all were BCT and TL-negative. All PCR results were also negative in human, animals and tsetse flies. Moreover, the sites were visited

by young people with an overall mean of age of 28.77 ± 1.21 years with 52.74% of male and 47.26% of female.

Evaluation of exposure levels of population to tsetse bites

Overall, the mean values of Ab responses related to the levels of exposure to tsetse bites were significantly different (Kruskal-Wallis: $\chi^2 = 15.819$, df = 6, $p = 0.01476$) regarding the villages included in this study (Fig. 2). These results showed that study population is low exposure to tsetse bites. Nevertheless, an inter-individual heterogeneity of Ab responses was observed with the highest response at 1.22 in Nyafogon village and the other responses ranging from 0 to 0.4. However, this highest responder was negative to the different biological tests.

Discussion

In recent decades, the mining sector of African countries in general and especially in West Africa has seen significant investments, leading in parallel to a considerable development of artisanal gold panning [28, 29, 30]. Burkina Faso is the 4th largest gold exporter in Africa in 2014 behind South Africa, Mali and Ghana [31]. Gold panning has also taken an important place in this country's economy [13]. However, gold panning has harmful consequences on the environment, agriculture, livestock, but also and specially on the health of the population, in particular the risks of cyanide, mercury, arsenic and selenium poisoning [12, 32]. In addition to these chemical risks, the presence of these gold panning sites in gallery forests, which are favorable for tsetse flies [8], exposes gold panners and populations living in the proximity of the gold sites to sleeping sickness. The objective of our study was to update the HAT data in Burkina Faso in the risk of the re-emergence context with the advent of artisanal gold mining.

The participation rate in our active medical survey was low and often difficult to evaluate due to the constant nomadism of the gold panners and the residents of the villages closer to gold sites, and their lack of interest in the study, which prevents from a real estimation of the population. An awareness phase had been carried out to avoid this disinterestedness. In our study, only 5 cases of positive CATT results were recorded while the parasitology and trypanolysis tests were negative. The PCR negativity confirms the trypanolysis results, meaning no case of HAT was detected during this study. The sensitivity of CATT is considered high but specificity limited [22]. This CATT positivity could be explained by cross-reactions with other parasites such as microfilaria found in the field [5]. These microfilariae are frequently detected in epidemiological activities in the field using BCT [5, 33]. In addition, the negative results of trypanolysis and BCT performed on the jugular vein blood of pigs did not show any contact with *T. b. gambiense*. Indeed, in 2015 a native case of sleeping sickness was detected in the village of Gouèra, which is part of our study area. The source of this infection could not be elucidated, but there were many hypotheses. One of them was a contamination from the animal reservoir [5]. This issue of the domestic / wild animal reservoir is still under study to better understand the epidemiological role of this reservoir in the transmission of HAT [1]. A recent study in Côte d'Ivoire has revealed the presence of *T. brucei sl* DNA by PCR and the highest TL positivity rate using *T. b. gambiense* specific variants in pigs [34]. Other domestic animals are also incriminated in this role of reservoirs of human trypanosomes such as cattle, sheep, goats, dogs [34, 35], horses and donkeys in Chad [36]. These studies highlight the importance of "One health" approaches to reach HAT elimination and contribute to African animal trypanosomosis control [37].

In addition, ELISA results showed significantly different levels of IgG Ab to the Tsgf1$_{18\text{-}48}$ peptide. Although tsetse are caught in the study area, the Ab responses thus revealed low human/tsetse contact which is a condition for the maintenance of HAT transmission.

Therefore, the presence of a human or animal reservoir in this area could be sufficient to maintain disease transmission. Since the decline in prevalence makes it difficult to detect new cases, the surveillance must be stepped up with the appropriate tools and strategies [2]. Thus, tools must be proposed to match to the epidemiological context for monitoring the elimination of HAT in order to react promptly and avoid a re-emergence as in the past [22].

Our study area is located in the agropastoral zones of Burkina Faso and includes historical HAT foci. The results showed that this area still harbors infected tsetse flies and populations from Côte d'Ivoire and Guinea, the countries most affected by HAT. This zone is quite close to Côte d'Ivoire, which increases the risk of HAT re-emergence due to significant population movements between this zone and endemic HAT foci in Côte d'Ivoire. Thus, population movements had always an impact on the evolution and spread of HAT in the past [3, 6]. In addition to gold panning, other minor activities considered at risk for HAT transmission like agriculture and livestock breeding, have been identified in our study area. Fishing, hunting, washing clothes, wood cutting, water supply in a watercourse, trade in rural areas and salt extracting were also mentioned to be activities that expose rural populations to HAT in other contexts [38, 39, 40]. A recent case-control study was found that artisanal diamond mining, which usually takes place in savannah areas that are generally infested with tsetse flies, was a risk activity for HAT in Mbuji Mayi, Democratic Republic of Congo [40]. Given to the proliferation of gold sites in infected tsetse areas, it is important to strengthen existing the health systems and to install new sanitary sentinel centres closer to the populations, by taking into account the gold mining. Moreover, the passive HAT surveillance system set up for low-endemicity countries such as Burkina Faso should be integrated into these new peripheral health centres. In these sanitary centres, regular training/awareness-raising for health workers and the population, could be offered to better control HAT. Finally, in order to reach

the objective of stopping transmission by the WHO in 2030, strategies adapted to each epidemiological context could be used [2].

In conclusion, this study conducted in the framework of proliferation of gold panning in tsetse-infested areas in southwestern Burkina Faso shows a low risk on the re-emergence of HAT. Although no case of HAT has been detected by parasitological tests, the Ab responses against a specific peptide of tsetse saliva reveal low human / tsetse contact. The results also showed that infected tsetse flies were caught in our study area. Moreover, activities like agriculture, breeding and gold panning, were practised in this area. The combination of these results highlights a risk, even low, of re-emergence of HAT in the southwestern Burkina Faso. Therefore, it is important to maintain passive surveillance and create new sanitary sentinel centres close to these artisanal gold sites, which host West African populations coming from Côte d'Ivoire and Guinea, which are of the most HAT affected countries.

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Declarations

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data supporting the conclusions of this article are included in this manuscript.

Code availability

Not applicable.

Authors' contributions

MBS, JK, SMK, ED, DD: conception of the research idea, study design, field activities, statistical analysis, data interpretation and drafting of the manuscript. CFAC: field activities, ELISA analysis, data interpretation and drafting of the manuscript. ES: entomological field activities, data interpretation and drafting of the manuscript. HI: designing of the study and drafting of the manuscript. HI, FC, VJ, ZB, AMGB: reviewing the manuscript. All authors who read and approved the final version of the manuscript.

Ethics approval

The research protocol was reviewed and approved by the ethical committee of CIRDES with the authorisation number 03-2016/CE-CIRDES. The study was carried out in collaboration with the HAT National Control Programme, the Regional Direction of Ministry of Livestock and the Regional Direction of Ministry of Health. Written consent was obtained from the authorities of the concerned villages and consent form has been signed by all people. All participants were informed about the objectives of the study in their local language. The collected samples were anonymised.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Legends to Figures and Tables

Fig. 1 Study area and sampling sites

Fig. 2 Distribution of antibody responses in villages including gold panning sites.

Asterisk "*" above the dot plot indicates the antibody response of individual that is 1.22.

460 **Table 1** Results of entomological surveys

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462 **Table 2** Characteristics of selected villages

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464 **Table 3** Results of medical survey in humans