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RESEARCH ARTICLE

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CHEMORESISTANCE OF *TRYPANOSOMA VIVAX* (KINETOPLASTIDA: TRYPANOSOMATIDAE) IN THE SUDANESE ZONE OF CÔTE D'IVOIRE

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ABSTRACT

African Animal Trypanosomoses constitute a major obstacle to the livestock development in sub-Saharan Africa. In response, trypanocides treatments are regularly administered to livestock. However, the uncontrolled and abusive use of these products has led to the emergence and expansion of trypanosome chemoresistance. The present study carried out in the departments of Korhogo and Ferkessédougou, in the Sudanese zone of Côte d'Ivoire, aims to assess the susceptibility of Trypanosoma vivax to diminazene aceturate and isometamidium chloride, the most commonly used molecules. Four stabilates were made from cattle fromlocalities of Napié (Sirikoli), Tioro (Nawalakaha), Kategué and Komborodougou and subjected to the in vivo resistance test on goats. With isometamidium, the experimental goats examined showed cases of post-treatment relapse. However, diminazene aceturate caused post-treatment relapses in some cases in experimental goats when used as a second line treatment against Trypanosoma vivax strains that have relapsed from isometamidium chloride. This result suggests that: (i) some of the Trypanosoma vivax strains tested developed resistance to isometamidium chloride; (ii) those found to be resistant to isometamidium seem to have also developed chemoresistance to diminazene aceturate. Whatever the situation, the present study conducted in Côte d'Ivoire highlighted additional cases of post-treatment failures using diminazene aceturate and isometamidium chloride against Trypanosoma vivax in West Africa.

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INTRODUCTION

African animal trypanosomoses (AAT) constitutes a major constraint to livestock development in sub-Saharan Africa. They threaten nearly 50 million cattle and 70 million small ruminants, over an area of about 10 million km^2 (Pattec, 2001; Pattec, 2000; De la Rocque *et al*., 2001; Kamuanga *et al*., 2005; Geerts & Holmes, 1998). As a result, treatment of livestock with trypanocides has become common practice in agropastoral farms, especially in West Africa (Touré, 1973; Trail *et al*., 1985; Ndoutamia *et al*., 1993; Kabamba & Malékani, 2017).

Farmers administrate prophylactic and curative treatment with trypanocides to animals on a regular (3 to 6 months) or occasional basis. Of the three products available (diminazene aceturate, isometamidium chloride and ethidium chloride/bromide), only diminazene aceturate (DA) and isometamidium chloride (ISM) are commonly used (Kabamba & Malékani, 2017; Leach & Roberts, 1981; Koné, 1999; Sones, 2001; Godfrey, 2010). However, the uncontrolled use of these sometimes-outdated products, often by unqualified persons, has resulted in the emergence and spread of drug resistance in trypanosomes (Talaki, 2008). Studies conducted in all regions of Africa to delimit and control this phenomenon showed that *T. congolense* and *T. vivax* have developed strains resistant to ISM and DA in 17 countries on the continent (Talaki, 2008; Geerts *et al*., 2001; Delespaux *et al*., 2008; Sow *et al*., 2013). In West Africa, however, the epidemiological situation of chemoresistance seems to be poorly understood. To overcome this deficiency, the "Centre International de Recherche-Développement sur l'Elevage en zone Subhumide" (CIRDES), in partnership with the Institute of Tropical Medicine (ITM), created in April 2009, the Epidemiological Surveillance Network on Resistance to Trypanocides and Acaricides in West Africa (RESCAO) currently active in eight countries (Benin, Burkina Faso, Côte d'Ivoire, Ghana, Mali, Niger, Nigeria, Togo). One of the main objectives is to improve the fight against AAT in this sub region by applying harmonized and adapted strategies (Anonyme, 2009; Vitouley *et al*., 2013). In Côte d'Ivoire, the socio-political crisis that occurred from 2002 to 2011 accentuated the conditions for poor application of trypanocides in livestock.

The picture of bad practices was mainly reflected in the use of the same product over a long period, the failure to follow the routes of administration and the dosage prescribed by the manufacturer, and finally, the use of falsified products. Thus, the "Laboratoire National d'Appui au Développement Agricole" (LANADA), which represents the State of Côte d'Ivoire within RESCAO, conducted activities to assess the situation of trypanosome chemoresistance in livestock in the Sudanese Region in the north of Cote d'Ivoire. For 4 months (September to December) in 2009, a total of 300 blood samples taken from cattle aged from 8 to 20 years old were analyzed. An examination of blood smears was performed to determine the trypanosomal prevalence. Then, trypanosome species were characterized using the PCR-RFLP (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism) technique, from buffy-coats collected on filter paper. Analyses revealed the existence of DA-resistant strains of *Trypanosoma congolense* in Boundiali, Niellé (Ferkessédougou) and Tioronandougou (Korhogo). The search for trypanocidal resistance markers, using the PCR-RFLP molecular diagnostic method, was only applicable to *T. congolense* species (Delespaux *et al*., 2008; Geysen *et al*., 2003; Delespaux *et al*., 2006). Therefore, the next step was to assess the chemoresistance of *T. vivax* to DA and ISM, using appropriate tests. The present study is conducted to evaluate the susceptibility of *T. vivax* to DA and ISM in potential foci of chemoresistance (Korhogo, Ferkessédougou) in the Sudanese zone of Côte d'Ivoire.

MATERIALS AND METHODS

Study area: The experiments were conducted in Korhogo (9°27'29"N/5°37'47"W) and Ferkessédougou (9°35'34"N/5°11'40"W) departments (Figure 1) recording long experience in the practice of agropastoral activities than other localities of Côte d'Ivoire (Le Guen, 2004). The climate is Sudanese type, characterized by average annual rainfall ranging from 1,200 mm to 2,500 mm, and by two alternating seasons: a rainy season from May to October with a peak in August and a dry season from November to April. This last season, is marked by the harmattan period that lasts from December to February (Eldin, 1971). The vegetation belongs to the Sudanese domain (Monnier, 1983), and is made up of open forests and a few gallery forests located along the rivers (Adjanohoun, 1965; Guillaumet & Adjanohoun, 1971). Sudanese zone extends within the Bandama River watershed, which, along with the Comoé, Sassandra, and Cavally rivers, forms the main rivers draining the Ivorian territory (Avenard *et al*., 1971). However, in the Sudanese zone, most of the water resources are provided by small dams (Anonyme (1992a,b), Da Costa *et al*., 1998).

Methods: The experiments were carried out in two phases. The first phase was conducted in the field in Sudanese zone of Côte d'Ivoire, to obtain *T. vivax* stabilates. The second phase was to evaluate the susceptibility of *T. vivax* to DA and ISM, was carried out at the Laboratory of Serology and Pathology of the CIRDES in Bobo-Dioulasso (Burkina Faso).

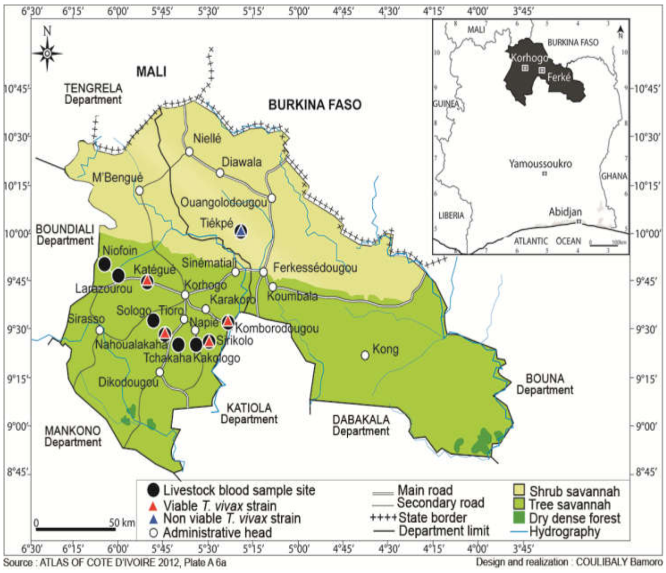


Figure 1. Study area and location of collection sites for T. vivax isolates

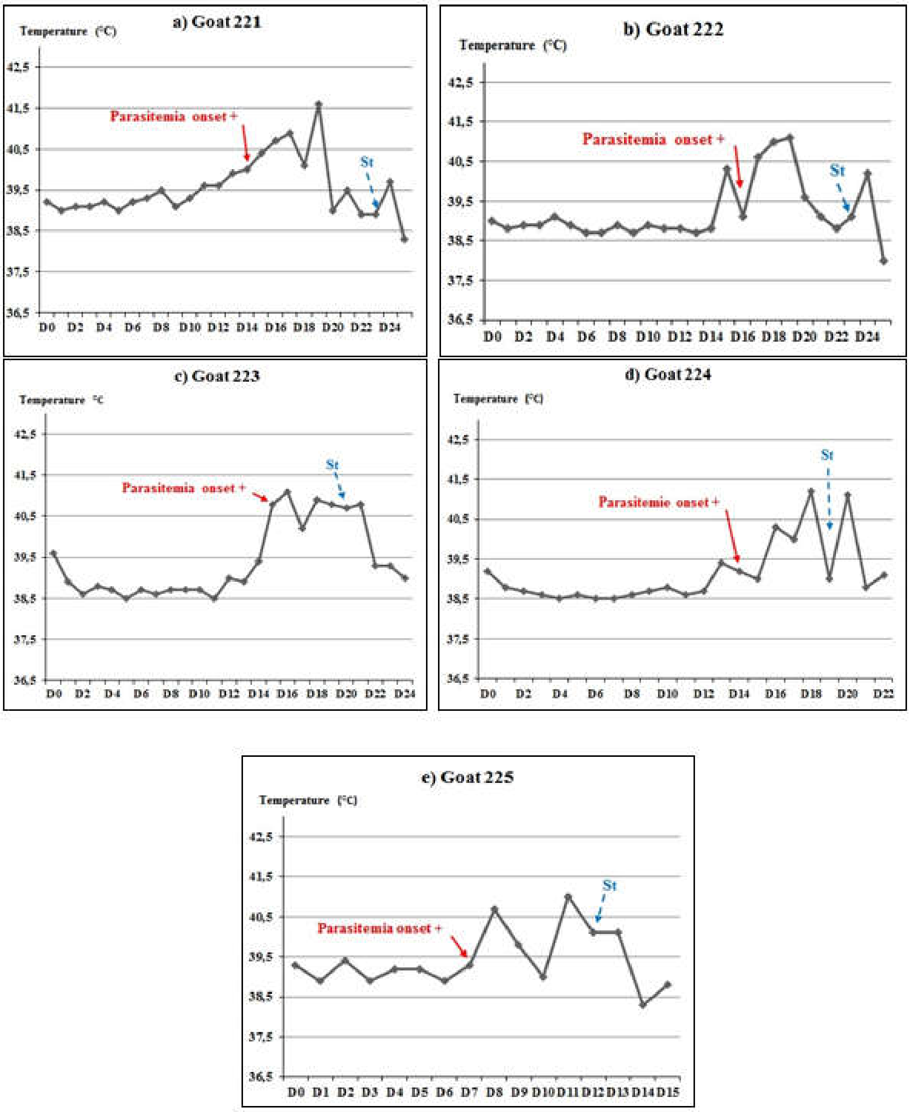
Field surveys

Sample size determination: The sample size was determined using the following standard formula (OIE, 2005):

$$n = \delta^2 * p * (1\text{-}p) * \frac{c}{i^2}$$

According to activity reports from LANADA's Korhogo Regional Laboratory (LRK), the overall trypanosomiasis prevalence estimated at 21.9%, varied from 7% to 29% in the departments of Korhogo and Ferkessédougou from 2008 to 2010. In addition, *T. vivax* accounted for nearly 10% of this overall prevalence. Thus, the objective of the sampling was to obtain a random sample of at least 300 cattle selected in a systematic way, to screen 30 of them for pathogenic trypanosome infestation.

Blood sampling from cattle: The surveys were conducted from February 20 to March 2, 2012, in 10 settlements (Tiekpé (Nambeguevogo), Niofoin, Kategué, Larazourou (Kategué), Sologo, Kakologo, Sirikoli, Nawalakaha, Tchakaha, Komborodougou). These localities are distributed in the sub-prefectures of Ferkessédougou, Niofoin, Korhogo, Napié, Tioro and Komborodougou (Figure I). They were selected based on results of a "Knowledge, Attitudes and Practices (KAP)" survey conducted by LANADA. The objective of this survey was to list the chemical molecules used as trypanocides by farmers in the region. Thus, the localities selected for the present study are those where farmers used to use AD (Berenil®, Survidim®, Trypanil®, Veriben®) and ISM (Trypamidium®) to fight AAT. The livestock screened represented both guarding (night groupings of cattle) and agropastoral (day groupings of animals for grazing) units. A total of 388 cattle were sampled out of 1,424 heads grouped into 22 herds (Table I). At least 30 animals were sampled per locality, except in Tchakaha, in the sub-prefecture of Tioro (Korhogo department), where the required conditions were not met to allow sampling of such many animals. Two types of blood samples were taken from each animal selected: capillary tubes and heparinized Vacutainer® tubes. Microscopic examinations of the blood collected from the capillary tubes were performed, after centrifugation, by the buffy coat examination technique (Murray, 1977). This examination revealed that, out of the 388 cattle collected, 13 were carriers of *T. congolense*, 5 of *T. vivax*, 3 of *T. brucei* and 55 were infected with microfilariae. Two cases of mixed trypanosome/microfilariae infections were found: one involved *T. congolense* and the other *T. vivax*. The most represented species was *T. congolense*.



Legend (St: preparation of *T. vivax* stabilate)

Figure 2. Evolution of the average daily temperatures of infected experimental goats and period of preparation of *T. vivax* stabilates

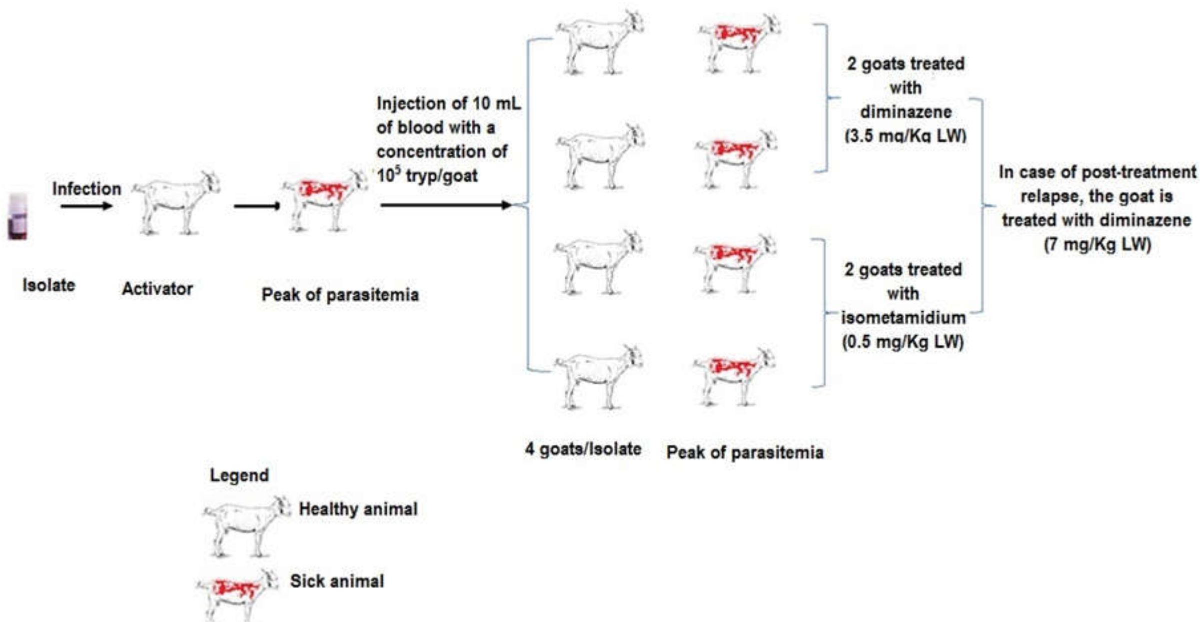


Figure 3. Diagram of the diminazene aceturate (DA) and isometamidium chloride (ISM) treatment protocol for experimental goats for one isolate

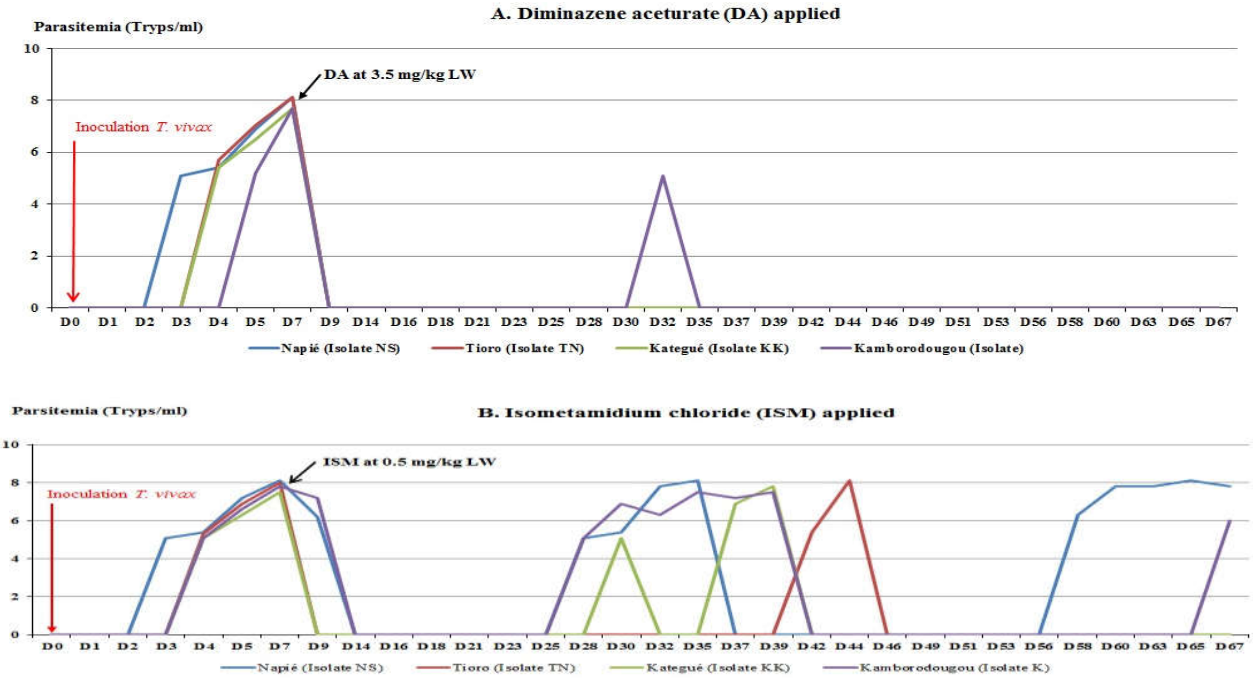
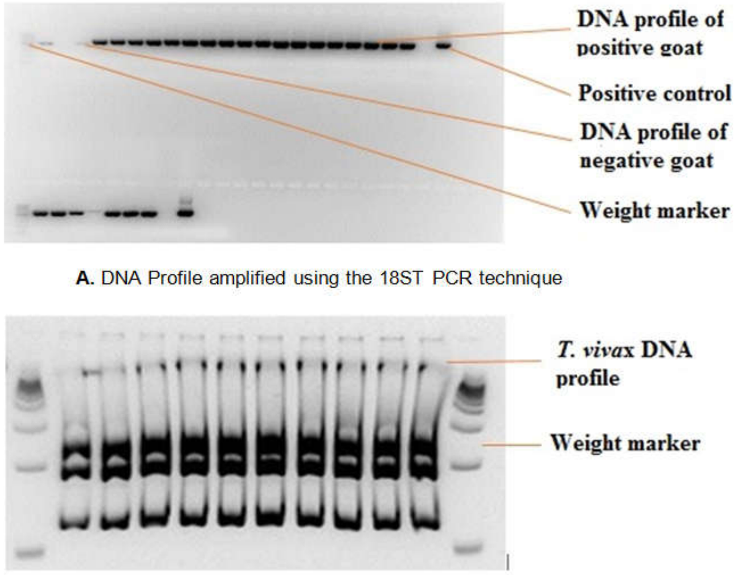


Figure 4. Variations in parasitemia in experimental goats inoculated with *T. vivax* and treated with trypanocides



A. DNA Profile amplified using the 18ST PCR technique

B. *T. vivax* profile characterized using RFLP technique

Figure 5. Results of the biomolecular diagnostic control from buffy coat of Sahelian goats used in the in vivo *T. vivax* resistance test

It infected cattle in 6 localities in Korhogo department (Nawalakaha, Kategué, Sologo, Kakologo, Sirkoli, Tchakaha). *T. congolense* was followed by *T. vivax* collected in 5 localities: 4 inin Korhogo (Nawalakaha, Kamborodougou, Kategué, Sirkoli) and one in Ferkessédougou (Tiekpé). Finally, *T. brucei*, the least represented species was collected from farms in three localities in Korhogo (Sologo, Kamborodougou, Kategué). For further processing, infected blood of the two species T. congolense and *T. vivax*, the most frequent in the study area, was selected. They were the main pathogens responsible for the most important trypanosomal diseases in the study area (Touré, 1977; Lefrançois *et al.*, 1998; Solano *et al.*, 1999; Bengaly *et al.*, 2002a; Bengaly *et al.*, 2002b; Acapovi, 2005; Djakaridja *et al.*, 2014; Acapovi-Yao *et al.*, 2016). Thus, blood samples in heparinized Vacutainer® tubes, taken from cattle infected with *T. congolense*, were used to make up samples collected on filter paper (Wattman n°1) using buffy coat. These samples were then sent to the CIRDES laboratory where they were used for molecular diagnosis of the chemoresistance of the species (*T. congolense*) by the PCR-RFLP method. As for the 5 samples taken from cattle infected by *T. vivax*, the corresponding heparinized Vacutainer® blood samples were used to prepare isolates for further investigations.

Collection of* T. vivax *isolates: Five goats aged 12 to 23 months (numbered 221, 222, 223, 224 and 225 respectively) were used to make *T. vivax* isolates. These goats came from the Sahel region of Dori, an arid climate area not favorable to the spread of AAT, located in the north of Burkina Faso and bordering the Republic of Niger. Upon receipt, each goat was bleached with DA in the commercial form Veriben® packaged at 3.5 mg/kg live weight (LW). Then, for three weeks, the bleached goats were fed and watered ad libitum. Then, they were treated with a broad-spectrum antibiotic (Limoxin-200 LA®) including 1ml/10kg BW. They were also dewormed with Oxfendazole® molecules (1 bolus/30kg). Finally, blood infected with *T. vivax*, collected in heparinized Vacutainer® tubes from the cattle, were inoculated respectively to the 5 Sahelian goats as they were collected (Goat 221: Tiekpé strain (Nambeguevogo); Goat 222:

Sirikoli strain (Napié); Goat 223: Nawalakaha strain (Tioro), Goat 224: Kategue (Korhogo); Goat 225: Komborodougou strain). The inoculation dose was 1 ml of infected *T. vivax* blood per experimental goat. From the day of inoculation noted D_0, the goats were followed regularly. Temperatures were recorded every day at 6am, 10am, 2pm and 6pm. Parasitemia was monitored every two days by observation of fresh blood taken from the ear. As soon as trypanosomes appeared in the blood of the goats, smears were taken to confirm the presence of *T. vivax*. At the peak of parasitemia, systemic blood was collected from the jugular vein in a vacutainer tube from each of the 5 experimental goats. Using a pipette, 1 ml of goat blood was then diluted in 111µl of 10% glycerol in a nunc tube. Each tube was referenced (date of preparation, trypanosome species, identification number of the goat) and indexed, then stored in liquid nitrogen (-176°C). All the samples conditioned (*T. vivax* isolates or stabilates) were transported to CIRDES to be tested for *T. vivax* susceptibility to DA and ISM. Figure 2 shows the temperature diagrams of the 5 experimental goats, indicating the periods of inoculation and preparation of *T. vivax* stabilates (Figure 2).

Laboratory experiments: At the CIRDES laboratory, the method adopted to assess the susceptibility of *T. vivax* to DA and ISM was the *in vivo* resistance test in goats (Sones *et al*., 1988; Eisler *et al*., 2001; Cuisance *et al*., 2003). This method was applied in three steps. First, trypanosome strains previously cryopreserved in liquid nitrogen at -176°C were tested for viability. Then, the strains that were found to be viable were amplified by inoculation to a susceptible host (Sahelian goat). Finally, the susceptibility of *T. vivax* was also evaluated *in vivo* in goats following experimental infections. The goats used were of the same age range (12 to 23 months) as the animals used to make the *T. vivax* isolates in Côte d'Ivoire. They also came from the same AAT-free arid climate zone of the Sahel, located in northern Burkina Faso. Again, upon receipt, the Sahelian goats were bleached with DA (Veriben® at 3.5 mg/kg LW), fed and watered *ad libitum*. Then, they were treated with a broad-spectrum antibiotic (Limoxin-200 LA® concentrated at 1 ml/10kg LW). In addition, before inoculation, the goats were dewormed with Oxfendazole® molecules, at the highest dose (1 bolus/30kg) capable of eliminating both strongyles and tapeworms. In the context of the *in vivo* resistance test at CIRDES, the goats used were placed under mosquito nets during the entire duration of the operations to avoid any external contamination. Finally, to ensure that these experimental goats were free of any trypanosomiasis, a control diagnosis was also carried out, first with buffy coat, then by the biomolecular method (PCR-RLFP).

Viability test of* T. vivax *strains: Viable *T. vivax* strains were identified under a binocular microscope, after mounting the stabilates between slides in glucose phosphorus buffer (GSP). Of the 5 *T. vivax* strains tested, 4 were found to be viable. These isolates were from Napié (Sirikoli): Isolat NS, Tioro (Nawalakaha): Isolat TN, Kategué: Isolat KK and Komborodougou: Isolat K. These stabilates have been retained for further operations.

Activation and amplification of viable T. vivax strains: The 4 viable *T. vivax* strains were activated from 4 goats called "isolate activator goats", numbered 1 to 4. For this purpose, the 4 viable isolates were inoculated on the same day (D_0) and at the same time, respectively to the 4 activating goats. From the 3rd day, daily blood samples were taken from each of the activating goats and analyzed by buffy coat to determine the parasitemia. Parasitemia was assessed based on the Herbert and Lumsden scale (Herbert & Lumsden, 1976). On this scale, the peak parasitemia is at least 6.9, i.e., the animal blood is at the concentration of $10^{6.9}$ trypanosomes per milliliter (Tryps/ml). The peak of parasitemia occurred on the 6th day after inoculation. At this stage, a molecular diagnostic control based on the PCR-RFLP technique was performed to ensure the effective presence of *T. vivax* in the blood of the activating goats. The hyperparasitic blood constituting the inoculate (E) was then collected respectively at the following doses: $E_1=10^{7.8}$ for isolate NS, $E_2=10^{7.5}$ for isolate TN, E_3=108.4 for isolate KK and $E_4=10^{8.1}$ for isolate K. This blood was diluted in glucose phosphorus buffer (GSP) to obtain an inoculum concentration of 10^5 Tryps/ml. The following dilution factor was considered:

$$Dilution\ factor = \frac{\text{concentration of hyperparasitic blood obtained}}{\text{inoculum concentration}}$$

Treatment of goats inoculated with* T. vivax *with DA and ISM: Each *T. vivax* inoculum obtained was used to infect a batch of 4 experimental goats at D_0, with 1 ml of inoculum per goat. From the 3rd day (D_3) onwards, the goats were monitored daily to determine peak parasitemia based on the Herbert and Lumsden scale (Herbert & Lumsden, 1976). The peak of parasitemia occurred on the7th day (D_7), with a variable rate depending on the batch of experimental goats and the type of trypanocide treatment administered. Thus, for the batch of goats inoculated with the NS isolate, the peak parasitemia was $10^{8.1}$ with both the DA and the ISM. For the batch corresponding to isolate TN, the peak was $10^{8.1}$ (DA) and 10^8 (ISM). For isolate KK, the peak was recorded at $10^{7.7}$ (DA) and $10^{7.5}$ (ISM). Finally, for the experimental goats inoculated with isolate K, the peak parasitemia was $10^{7.7}$ with DA and $10^{7.8}$ with ISM. At this stage (D_7), each hyperparasitized goat received a trypanocidal treatment. The products used were DA (Veriben®, CEVA SANTE ANIMALE, France) at a dose of 3.5 mg/kg LW and ISM (Veridium™, CEVA SANTE ANIMALE, France) at a concentration of 0.5 mg/kg LW. Out of the 4 experimental goats, two were treated with DA and the other two with ISM. Thus, with each trypanocide, 8 experimental goats were treated (Figure 3), making a total of 16 goats used in the experiments. The body parameters (temperature, weight) of the animals were recorded to determine the prepatent periods. The results were evaluated at D_{28} and D_{56}. Then, blood samples were taken to assess parasitemia. At the first post-treatment relapse, the affected goats were treated again, but this time with DA at a dose of 7 mg/kg LW. No further treatment was given after this step, even in case of a second relapse. Before administering each trypanocidal treatment at the peak of parasitemia, a diagnostic control was carried out to ensure that the experimental goats used were only infected with *T. vivax*. Thus, the actual presence of the parasite in the blood of the goats was demonstrated by buffy coat. *T. vivax* was then characterized using the biomolecular method based on the PCR-RFLP test.

Biomolecular control techniques

18ST PCR techniques: DNA was extracted with Chelex-100 (10%) in a 0.5% PBS-saponin solution. The extraction of genetic material was done from buffy coats from the blood of parasitemic goats collected on Whatman paper. The extracted DNA was amplified in a master mix including yellow sub, distilled water, 5X buffer, MbCl, dNTP, primers and finally Taq polymerase. The amplification was performed using a thermal cycler in the presence 5 µl of known *T. vivax* DNA used as control. The operation was performed in two steps. The first step was performed in 20µl of master mix and 5µl of Chelex supernatant (DNA solution). The pair of primer used included 18STnF2 (CAA-CGA-TGA-CAC-CCA-TGA-ATT-GGG-GA) as forward primer and 18STnR3 (TGC-GCG-ACC-AAT-AAT-TGC-AAT-AC) as reverse primer. The second step of the amplification reaction used a solution composed of 24.5µl of the master mix and 0.5µl of Chelex supernatant. The pair of primer included the forward primer from the first step and 18STnR2 (GTG-TCT-TGT-TCT-CAC-TGA-CAT-TGT-AGT-G) as reverse primer. The amplification products were then subjected to agarose gel electrophoresis (2%) stained with ethidium bromide (0.5µg/ml), in 2µl TAE (Tris-Acid Boric-EDTA) buffer. The result of this migration was read from ultraviolet photography.

RLFP technique: The protocol is based on the digestion of a target DNA by one or more restriction enzymes specific to the restriction sites carried by this DNA. After electrophoresis, the separated fragments are hybridized with a probe DNA, often from genomic or complementary DNA libraries (Geysen *et al*., 2003; Delespaux *et al*., 2003; García *et al*., 2014). In the present study, the RLFP assay was performed using the amplified DNA bands, which migrated on the

18ST PCR gel. The genetic material was digested with the restriction enzyme Msp1 (Westburg; Reference: R0106S). The operation was performed in a reaction mixture defined by the manufacturer. This mixture, with a total volume of 40 µl, included 10 to 15µl of PCR product, 5U of restriction enzyme, and the volume was adjusted with sterile distilled water. Each of the digesting product was then subjected to agarose gel electrophoresis (2%) in 0.5µg/ml ethidium bromide.

Data processing and analysis: The following parameters were determined: (i) post-treatment variations in body weight, (ii) prepatent periods or periods between inoculation of goats and the appearance of parasites in the blood of inoculated animals, (iii) relapse interval which is the time interval between the reappearance of trypanosomes in an animal after its first trypanocidal treatment, and, (iv) hematocrit. Hematocrit was determined using a hematimetric cell, after centrifugation of blood collected in capillary tubes (12,000 rpm for 5mm). It is the ratio expressed as a percentage of the volume of red blood cells related to the total volume of blood. Finally, the mean evolution of hematocrit or Packed Cell Volume (ΔPCV) as well as that of weight (ΔMW) were calculated according to the belowing formulas (Agarwal, 1996):

$$\Delta PCV = \left[\frac{PCV2 - PCV1}{PCV1}\right] * 100\ ;\quad \Delta MW = \left[\frac{Mw2 - Mw1}{Mw1}\right] * 100$$

Post-treatment relapse rates, represented by aparasitemic animals that subsequently become parasitologically positive after treatment, were used to characterize chemoresistance. Indeed, such an isolate is declared chemoresistant to the dose of trypanocide administered when at least 20% of the inoculated animals present a post-treatment relapse, which corresponds to at least 1 relapse for a total of one to four treated cattle (Eisler *et al*., 2001). Statistical analyses focused on the comparison of means for the parameters of weight, hematocrit and prepatent periods. These analyses were performed using the Student's t test with Excel and STATISTICA version 7.1 software. The confidence interval and statistical significance level were 95% and 5%, respectively. Comparisons were also made with the Pearson 2 test for values greater than 5 or with the Fisher test at the 5% threshold for values less than 5.

These goats had become aparasitemic whereas at the time of treatment on D_7, they had parasitemia levels ranging from $10^{7.5}$ to $10^{8.1}$ Tryps/ml. For these 8 goats treated with DA, no post-treatment relapse was observed until the end of the experiments. In contrast, with ISM, 4 of the 8 goats showed relapse after the first treatment with NS, KK, K and TN isolates, respectively. In the second period, only the 2 goats that received the NS and K isolates, again showed post-treatment relapse (Table IV, Figure 4). Thus, after the first treatment of all the goats at D_7, the administration of the DA in second intention concerned only goats already treated with the ISM. These goats, which had become aparaemic after the first treatment, became parasitaemically positive again: D_{35} for those inoculated with the NS isolate (Parasitemia: $10^{8.1}$ Tryps/ml), D_{39} with the KK (Parasitemia: $10^{7.8}$ Tryps/ml) and K (Parasitemia: $10^{7.5}$ Tryps/ml) isolates, and finally, D_{44} for the goats inoculated with the TN isolate (Parasitemia: $10^{8.1}$ Tryps/ml). The second treatment was administered on a case-by-case basis as the relapse occurred. A total of 28 blood samples were used for molecular diagnostic control. Indeed, the 4 activating goats were sampled twice: before and after their inoculation giving 8 samples. In addition, before their trypanocidal treatments, the experimental goats were sampled in two stages. First, all 16 goats were sampled at D_7, then the 4 experimental goats that had relapsed at D_{35} (goat inoculated with the NS isolate), D_{39} (the 2 goats that received the KK and K isolates) and D_{44} (goat with the TN isolate) respectively. On analysis, 18ST PCR revealed that the 4 activating goats collected before inoculation were indeed free of trypanosomes. However, these 4 goats after being inoculated with *T. vivax*, as well as the 20 samples taken from the experimental goats at the different peaks of parasitemia, were positive (Figure 5A). During the RFLP test, the Msp1 restriction enzyme used has already been used to make a clear distinction in characterizing *T. congolense, T. brucei and T. vivax* (20). Of note, all three species had been observed in cattle at the time of preparation of *T. vivax* isolates in the Sudanese zone of Côte d'Ivoire. However, analysis of the RFLP results showed that they were not present in the blood of uninoculated Sahelian goats or in the blood of goats that had been inoculated during the in vivo test. In those animals, *T. vivax* was the only species that was characterized by RFLP, indicating that isolates made from cattle blood in Côte d'Ivoire were infected only with this pathogen (Figure 5B).

Table 1. Study sites, herd sizes and livestock sampled

Department	Sub-prefectures	Localities (Study site)	Number of herds	Number of livestock	Number of bovines sampled
Ferkessédougou	Ferkessédougou	Tiékpé (Nambeguevogo)	2	125	30
Korhogo	Niofoin	Niofoin	2	264	61
	Korhogo	Larazourou (Kategue)	3	165	41
		Kategue	3	177	46
		Sologo	4	205	60
	Napié	Kakologo	1	65	30
		Sirikoli	1	55	30
	Tiorio	Nawalakaha	3	190	40
		Tchakaha	1	55	10
	Komborodougou	Komborodougou	2	123	30
Total			22	1424	388

RESULTS

Analysis of weight changes shows a drop in weight in experimental goats after *T. vivax* inoculation, regardless of the trypanocidal treatment applied. However, there was a recovery of weight at D_{56} in animals infected with isolate K and treated with DA (ΔMW = 0.02 kg) (Table II). Like the weight, hematocrit values decreased during the experimental period. Hematocrit values were less than 25% at D_{28} in goats inoculated with TN or K strains and treated with DA. The same was true at D_{56} with goats treated with ISM, for all strains of *T. vivax* (Table III). The prepatent periods ranged from 4 to 8 days. The average per isolate was 5 to 6 days with the overall average for all samples estimated at 5.5 days. By the first post-treatment check (D_{19}) after inoculation of the experimental goats, parasitaemia had dropped significantly in all goats treated with DA.

DISCUSSION

For each *T. vivax* stabilate tested, only two replicates were performed, whereas the protocol of Eisler *et al*. (2001) suggests the use of a minimum of three or, preferably, six animals. Errors could therefore have resulted from the individual responses of Sahelian goats inoculated with the same *T. vivax* isolate. Indeed, these responses may be different depending on the trypanocidal treatment (DA or ISM) administered (Ndoutamia *et al*., 1993; Koné, 1999; Hawking, 1963; Peregrine *et al*., 2001). Nevertheless, despite these methodological shortcomings related to sampling, the study found that with ISM at a dose of 0.5 mg/kg LW, the experimental goats that were screened showed cases of post-treatment relapse. This result was observed in 50% of the cases, a rate well above 20%.

Table 2. Comparison of variations in mean weights (ΔMW) in kg at D_{28} and D_{56}, of experimental goats treated with trypanocides

Locality	Diminazene acéturate (DA)							Isometamidium chloride (ISM)						
	28 days post-treatment (D_{28})			56 days post-treatment (D_{56})			Statistical value (p)	28 days post-treatment (D_{28})			56 days post-treatment (D_{56})			Statistical value (p)
	Mw	Standard deviation	ΔMw	Mw	Standard deviation	ΔMw		Mw	Standard deviation	ΔMw	Mw	Standard deviation	ΔMw	
Napié (Sirikoli)	14,4	±3,394	-0,12	14,9	±2,404	-0,12	1	13,9	±0,707	0,02	13,8	±0,0	0,01	0,927762
Tioro (Nawalakaha)	14,1	±2,121	-0,11	13,6	±3,111	-0,14	0.692459	11,6	±0,0	-0,32	12,6	±0,0	-0,26	0,589942
Kategué (Korhogo)	10,5	±0,141	-0,2	11,6	±0,0	-0,11	0,253477	12,0	±2,828	-0,15	14,0	±0,0	-0,15	1
Komborodougou	12,9	±2,687	-0,17	13,5	±4,101	0,02	P<0.05	12,6	±0,0	-0,19	13,8	±0,0	-0,11	0,475616

Table 3. Comparison of variations in mean (%) hematocrit or Packed Cell Volume rate (ΔPCV) at D_{28} and D_{56} in experimental goats treated with trypanocides

Localité	Diminazene acéturate (DA)							Isometamidium chloride (ISM)						
	28 days post-treatment (D_{28})			56 days post-treatment (D_{56})			Statistical value (p)	28 days post-treatment (D_{28})			56 days post-treatment (D_{56})			Statistical value (p)
	PCV	Standard deviation	ΔPCV	PCV	Standard deviation	ΔPCV		PCV	Standard deviation	ΔPCV	PCV	Standard deviation	ΔPCV	
Napié (Sirikoli)	27	±2,828	-0,17	25	±0,0	-0,23	1	20	±1,414	-0,18	18	±0,0	-0,27	0,927762
Tioro (Nawalakaha)	21	±2,828	-0,21	25	±0,0	-0,06	0.692459	29	±0,0	0,12	15	±0,0	-0,42	0,589942
Kategué (Korhogo)	30,5	±4,950	-0,15	29,5	±0,707	-0,18	0,253477	28,5	±0,707	-0,12	27	±0,0	-0,22	1
Komborodougou	22,5	±0,707	-0,15	24,5	±0,707	-0,08	P<0.05	25,5	±0,707	-0,15	21	±0,0	-0,3	0,475616

Table 4. Prepatent periods and relapse intervals of goats treated with trypanocides

Locality	Nº Goat	PREPATENT PERIOD			POST-TRAITMENT RELAPSE	
		No. of Days	App ± Str D (Days)	Tapp ± Str D (Days)	After 1st treatment	After 2nd treatment
Napié (Sikoli)	1AD1	4	6,0±2,309	5,5±0,408	A	A
	1AD2	8			A	A
	1ISM1	8			A	A
	1ISM2	4			D_{28}	D_{56}
Tioro (Nawalakaha)	2AD1	5	5,0±0,00		A	A
	2AD2	5			A	A
	2ISM1	5			D_{42}	A
	2ISM2	5			A	A
Kategué (Korhogo)	3AD1	6	5,5±0,577		A	A
	3AD2	5			A	A
	3ISM1	6			D_{30}	A
	3ISM2	5			A	A
Komborodougou	4AD1	6	5,5±0,577		A	A
	4AD2	6			A	A
	4ISM1	5			D_{28}	D_{67}
	4ISM2	5			A	A

App ± Str D : Average prepatent period ± Standard deviation ; Tapp ± Str D : Total average prepatent period ± Standard deviationDA : Goats treated with diminazene aceturate; ISM : Goats treated with isometamidium chloride A : Aparasitemia D_n : n^{th} day

As a reminder, as of the experimental protocol applied, this rate of 20% is usually recognized as an indicator of treatment failure, since the inoculated isolate is resistant to the trypanocide administered (Eisler *et al*., 2001). Thus, the post-treatment failures with ISM in experimental goats suggest that the *T. vivax* strains used have developed resistance to this trypanocide. With respect to DA, at the dose used (3.5 mg/kg LW) in the first line, the product seems to have been rather effective against *T. vivax* strains. However, when applied as a second-line treatment against *T. vivax* strains that showed resistance to ISM treatment, albeit at twice the dose (7 mg/kg LW) of the first-line treatment, DA caused post-treatment relapse in some cases. Apparently, the *T. vivax* strains that relapsed after ISM treatment also seem to have lost their susceptibility to DA at the same time. In general, this phenomenon is observed in cases of acquired resistance (Mungube, 2010). In any case, observations made in the field seem to support the thesis that poor application of trypanocides in the herds may be one of the main causes of post treatment relapse to DA and ISM in the study area. Indeed, in 21 of the 22 herds screened during the experiments, the herdsman was the treating agent. Only in the 4 herds in the Tioro locality treatments were administered by a private veterinary technician. It stems from these observations that the cases of post-treatment relapse, particularly with ISM, recorded in the study area, were also highlighted in Tioro, where the cattle were nevertheless monitored by a trained technician. The situation seems to be much more worrying for the entire Sudanese zone because, from now on, the use of trypanocides against AAT appears to be a risk undertaking. It's worthy to indicate that, the cases of post-treatment relapses at AD and ISM thus highlighted in the Sudanese zone of Côte d'Ivoire through the present study, in addition to those already described elsewhere in Africa, show the extent of the phenomenon of drug resistance of trypanosomes on the continent. Therefore, new approaches are sought to combat AAT. In this perspective, in general, to overcome difficulties related to the chemoresistance, substitute chemical molecules were often used (TOURÉ, 1973). However, given the scale of the phenomenon, an integrated approach, combining chemical treatments and vector control is increasingly recommended (Uilenberg, 1996 ; PATTEC, 2004 ; Komono *et al*., 2011).

CONCLUSION

The *T. vivax* strains considered seem to have developed resistance to ISM at the dose of 0.5 mg/kg LW. The results obtained with DA are contradictory. The product seems to have been effective in the first line against *T. vivax* at the dose of 3.5 mg/kg LW. However, when used as a second-line treatment, *T. vivax* strains that were resistant to ISM appeared to become resistant to DA. Thus, the present study has highlighted additional cases of post-treatment failure of DA and ISM against *T. vivax* in Côte d'Ivoire. With these cases added to those already reported in West Africa as well as in the rest of the continent, the current efficacy of the use of trypanocides against TAA now seems unreliable. New methods are therefore being sought. Among the main strategies advocated, including the use of alternative products, preferences tend towards an integrated approach combining the use of trypanocidal products and vector control.

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LIST OF ABBREVIATIONS

AAT: Animal African Trypanosomosis
BCT: Buffy Coat Technique
CIRDES: Centre International de Recherche-Développement sur l'Elevage en
zone Subhumide
DA: Diminazene Aceturate
DNA: Desoxyribonucleic Acid
GSP: Glucose Phosphorus Buffer
ISM: Isometamidium Chloride
ITM: Institute of Tropical Medicine
K: Isolate from Kategué
KAP: Knowledge, Attitudes and Practices
KK: Isolate from Komborodougou
LANADA: Laboratoire National d'Appui au Développement Agricole
NS: Isolate from Napié (Sirikoli)
LW: Live Weight
PATTEC: Pan African Tsetse and Trypanosomiasis Eradication Campaign
PCR: Polymerase Chain Reaction
PCV: Packed Cell Volume
RESCAO: Réseau d'Epidemio-Surveillance de la Chimiorésistance aux
trypanocides et acaricides en Afrique de l'Ouest
RFLP: Restriction Fragment Length Polymorphism
T. brucei: *Trypanosoma brucei*
T. congolense: *Trypanosoma congolense*
T. vivax: *Trypanosoma vivax*
TN: Isolate from Tioro (Nawalakaha)

REFERENCES

Acapovi GL (2005). Identification et bioécologie des tabanidés, vecteurs mécaniques potentiels de la trypanosomose bovine dans les régions de savanes en Côte d'Ivoire (Odienné et Korhogo). Université de Cocody, Thèse de doctorat n°435, Abidjan, 137pp.

Acapovi-YAO GL, Cissé B, Zinga Koumba CR, Mavoungou JF (2016). Infections trypanosomiennes chez les bovins dans des élevages de différents départements en Côte d'Ivoire. *Revue Médecine Vétérinaire*, 167(9-10): 289-295.

Adjanohoun F (1965). Comparaison entre les savanes côtières de Côte d'Ivoire et du Dahomey. *Annale de l'Université d'Abidjan*, 1: 41-60.

Agarwal BL (1996). Basic statistics. New Age International, 3rd ed., 703pp.

Anonyme (1992a). Valorisation du potentiel piscicole des barrages hydro-agro-pastoraux du Nord de la Côte d'Ivoire. Rapport préliminaire, Projet "Petits Barrages". Convention IDESSA/CRDI 3-P-89-0215, Côte d'Ivoire, 181pp.

Anonyme (1992b). Inventaire des barrages hydro-agricoles et à autres vocations existant en Côte d'Ivoire. Direction et Contrôle des Grands Travaux (DCGTx). Rapport multigr., Côte d'Ivoire, 57pp.

Anonyme (2009). Charte de fonctionnement du Réseau d'épidémiosurveillance de la résistance aux trypanocides et aux acaricides en Afrique de l'Ouest (RESCAO) – 17 juin 2009.

Avenard JM, Eldin M, Girard G, SIRCOULON J, Touchebeeuf P, Guillaumet JL, Adjanohoun E, Perraud A (1971). Le milieu naturel de Côte d'Ivoire. Mémoire ORSTOM, 50, Paris, 391pp.

Bengaly Z, Sidibé I, Boly H, Sawadogo L, Desquesnes M (2002a). Comparative pathogenicity of three genetically distinct *Trypanosoma congolense*-types in inbred Balb/c mice. *Veterinary Parasitology*, 105(2): 111-118.

Bengaly Z, Sidibé I, Ganaba R, Desquesnes M, Boly H, Sawadogo L (2002b). Comparative pathogenicity of three genetically distinct types of *Trypanosoma congolense* in cattle: clinical observations and haematological changes. *Veterinary Parasitology*, 108(1): 1-19.

Cuisance D, Itard J, Solano P, Desquesnes M, Frézil JL Authié E (2003). Trypanosomoses : Méthodes de lutte. *In* : Principales maladies infectieuses et parasitaires du bétail : Europe et Régions chaudes. Tome 2 : Maladies bactériennes, mycoses, maladies parasitaires. *AUPELF-UREF/TEC & DOC* (Lavoisier), Paris, 1695-1724.

Da Costa KS, Traoré K, Tito De Morais L (1998). Effort de pêche et production exploitée dans les petites retenues du Nord de la Côte d'Ivoire. *Bulletin français de la pêche et de la pisciculture*, 71(348): 65-78.

De La Roque S, Michel JF, Cuisance D, De Wispelaere G, Solano P, Augusseau X, Arnaud M, Guillobez S (2001). Du satellite au microsatellite. Le risque trypanosomien. Une approche globale pour une décision locale. CIRAD, France, Montpellier, 151pp.

Delespaux V, Ayral F, Geysen D, Geerts S (2003). PCR-RFLP using Ssu-rDNA amplification: Applicability for the diagnosis of mixed infections with different trypanosome species in cattle. *Veterinary Parasitology*, 117: 185-193.

Delespaux V, Chitanga S, Geysen D, Goethals A, Van Den Bossche P, Geerts S (2006). SSCP analysis of the P2 purine transporter TcoAT1 gene of *Trypanosoma congolense* leads to a simple PCR-RFLP test allowing the rapid identification of diminazene resistant stocks. *Acta Tropica.*, 100(1-2): 96-102.

Delespaux V, Geysen D, Van Den Bossche P, Geerts S (2008). Molecular tools for the rapid detection of drug resistance in animal trypanosomes. *Trends in Parasitology,* 24(5): 236-242.

Djakaridja B, Yao-Kouassi P, Gragnon Biégo G, Acapovi-Yao G, Mavoungou JF, N'Goran Kouakou E (2014). Situation épidémiologique des hémoparasites des bovins dans deux zones d'élevage de la Cote d'Ivoire : cas des anciennes régions des Savanes et de la vallée du Bandama. *Revue de Médecine Vétérinaire*, 165(9-10): 297-303.

Eisler MC, Brandt J, Bauer B, Clausen PH, Delespaux V, Holmess PH, Ilemobade A, Machila N, Mbwambo H, McDermott J, Mehlitz D, Murilla G, Ndung'u JM, Pérégrine AS, Sidibé I, Sinyangwe L, Geerts S (2001). Standardised tests in mice and cattle for the detection of drug resistance in tse-tse transmitted trypanosomes of African domestic cattle. *Veterinary Parasitology*, 97: 171-182.

Eldin M (1971). Le climat. *In* : Le milieu naturel de la Côte d'Ivoire. *Mémoire ORSTOM*, 50, 73-108.

García LT, Ardila YA, RincÓn D, Durán C, Aguilar JR (2014). A new PCR-RFLP for species-specific diagnosis of South American animal trypanosomiasis. *American Journal of Animal and Veterinary Sciences*, 9(2): 128-136.

Geerts S & Holmes PH (1998). Drug management and parasite resistance in bovine trypanosomiasis in Africa. PAAT Technical Sciences Series, n° 1, FAO, Rome, 31pp.

Geerts S & Holmes PH, Diall O, Eisler C (2001). African bovine trypanosomiasis: the problem of drug resistance, *Trends in Parasitology*, 17: 25-28.

Geysen D, Delespaux V, Geerts S (2003). PCR/RFLP Using Ssu-rDNA amplification as an easy method for species-specific diagnosis of *Trypanosoma* species in cattle. *Veterinary Parasitology*, 110: 171-180.

Godfrey K (2010). Comparative study of tsetse and trypanosomosis control methods in Kasese District, Makerere University, Kampala (Ouganda), 78pp.

Guillaumet JL, Adjanohoun F (1971). La végétation de la Côte d'Ivoire. *In* le milieu naturel de la Côte d'Ivoire. *Mémoire ORSTOM.*, 50, 157-263.

Hawking F (1963). Drug resistance of *T. congolense* and other trypanosomes to quinapyramine, phenanthridines, Berenil and other compounds in mice. *Annals of Tropical Medicine and Parasitology*, 57: 262-282.

Herbert WJ, Lumsden WHR (1976). *Trypanosoma brucei*: A rapid "matching" method for estimating the host's parasitemia. *Elsevier*, *Experimental Parasitology*, 10(3): 427-431.

Kabamba MW, Malekani J (2017). Épidémiologie de la trypanosomose animale africaine (TAA) à l'Ouest de la République Démocratique du Congo (RDC). *Revue Marocaine des Sciences Agronomiques et Vétérinaires*, 5(2): 108-111.

Kamuanga M, Hamadou S, Bouyer J, Yao Y, Sidibé I, Kaboré I (2005). Comment pérenniser les acquis de la lutte antivectorielle ? Fiche technique n°14, CIDES/CIRAD, France, 8pp.

Komono BD, Koffi M, Coulibaly B (2011). Problématique de la lutte contre la maladie du sommeil. *Médecine d'Afrique Noire*, 58(6): 315-326.

Koné PS (1999). Comparaison des tests in vivo: test sur souris et test sur bovins pour la detection de la résistance vis-à-vis de l'isométamidium chez *Trypanosoma congolense*. Master's degree in Tropical Animal Health, n°84, Institut Prince Leopold Anvers, Belgique, 49pp.

Le Guen T (2004). Le développement agricole et pastoral du Nord de la Côte-d'Ivoire : problèmes de coexistence. Les Cahiers d'Outre-Mer, 226-227.

Leach TM & Roberts CJ (1981). Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the eastern hemisphere. *Pharmacology and Therapeutics*, 13: 91-147.

Lefrançois T, Solano P, De La Rocque S, Bengaly Z, Reifenberg JM, Kaboré I, Cuisance D (1998). New epidemiologicalfeatures on animal trypanosomosis by molecular analysis in the pastoral zone of Sidéradougou, Burkina Faso. *Molecular Ecology,* 7: 897–904.

Monnier Y (1983). Végétation *In* "Les atlas Jeune Afrique. Côte d'Ivoire". Edition Jeune Afrique, Paris, 16-18.

Mungube EO (2010). Management of trypanocidal drug resistance in cattle in identified chemoresistance hot spots in the administrative District of Sikasso, south- east Mali, Thesis of a doctorat degree in Veterinary Medecine, Berlin, 192p.

Murray M, Murray PK, McIntryre WLM (1977). An improved parasitological technique for the diagnosis of African trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 71(4): 325-326.

Ndoutamia G, Moloo SK, Murphy NB, Pérégrine AS (1993). Derivation and characterisation of a quinapyramine resistant clone of *T. congolense*. Antimicrob. *Agents Chemother*, 37: 1163-1166.

OIE (2005). Méthodes d'échantillonnage. *In* : Manuel terrestre de l'OIE, 9, CIRAD, France, Montpellier, 3-15.

PATTEC (2000). Pan African tsetse and trypanosomosis Eradication campaign (PATTEC). A continental plan of action for the eradication of Tsetse and trypanosomosis. Proceedings of the OAU Pathway for the PATTEC Initiative, Dec. 7-16, Nairobi, Kenya, 39pp.

PATTEC (2001). Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC): Enhancing Africa's Prosperity: Plan of action. Organization of African Unity, Addis Ababa, 31pp.

PATTEC (2004). Turning decisions into action. PATTEC newsletter, U.A., Addis Ababa, 29pp.

Pérégrine AS, Sidibe I, Sinyangwe L, Geerts S (2001). Standardised tests in mice and cattle for the detection of drug resistance in tse-tse transmitted trypanosomes of African domestic cattle. *Veterinary Parasitology*, 97: 171-182.

Solano P, Michel JF, Lefrançois T, De La Rocque S, Sidibé I, Zougrana A, Cuisance D (1999). Polymerase chain reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkina Faso. *Veterinary Parasitology*, 86(2): 95-103.

Sones K (2001). Pharmaceutical companies: partners or enemies? *ICPTV Newsletter*, 3: 19-21.

Sones RK, Njogu AR, Holmes PH (1988). Assessment of sensitivity of *Trypanosoma congolense* to isometamidium chloride: a comparison of tests using cattle and mice. *Acta Tropica*, 45: 153-164.

Sow A, Sidibé I, Bengaly Z, Marcotty T, Séré M, Diallo A, Vitouley HS, Nébié RL, Ouédraogo M, Van Den Bossche P, Van Den Abbeele J, De Deken R, Delespaux V (2013). Field detection of resistance to isometamidium chloride and diminazene aceturate in *Trypanosoma vivax* from the region of the Boucle du Mouhoun in Burkina Faso. *Elsevier*, 8(1-2): 105-111.

Talaki E (2008). Etude de la résistance des trypanosomes à l'isométamidium et au diminazène dans la zone cotonnière de l'Afrique de l'Ouest (Mali - Guinée - Burkina Faso), Thèse de Doctorat de l'Université polytechnique de Bobo-Dioulasso, Burkina Faso, 198pp.

Touré S (1977). La trypanotolérance. Revue des connaissances. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux*, 30: 157-174.

Touré SM (1973). Les trypanocides et leur utilisation en médecine vétérinaire. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux,* 26(4): 113a-122a.

Trail JCM, Murray M, Sones K, Jibbo JMC, Darkin J, Ligth D (1985). Boran cattle maintained by chemorprophylaxis under trypanosomiasis risk. *Journal of Agricultural Science*, 105: 147-166.

Uilenberg G (1996). Lutte intégrée contre les parasitoses animales tropicales. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux*, 49(2): 124-129.

Vitouley HS, Bengaly Z, Adakal H, Sidibé I, Van Den Abbeele J, Delespaux V (2013). Réseau d'EpidémioSurveillance de la Chimiorésistance aux trypanocides et aux acaricides en Afrique de l'Ouest (RESCAO). *Tropicultura*, 31(3): 205-212.

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