

**Field detection of resistance to isometamidium chloride and diminazene aceturate in  
*Trypanosoma vivax* from the Region of the Boucle du Mouhoun in Burkina Faso**

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## Abstract

A longitudinal study assessed the chemoresistance to isometamidium chloride (ISM) and diminazene aceturate (DA) in the region of the Boucle du Mouhoun in Burkina Faso. A preliminary cross-sectional survey allowed the identification of the 10 villages with the highest parasitological prevalences (from 2.1% to 16.1%). In each of these 10 villages, two herds of approximately 50 bovines were selected, one being treated with ISM (1mg/kg b.w.) and the other remaining untreated as control group. All animals (treated and untreated herds) becoming infected were treated with DA (3.5mg/kg b.w.). In total, 978 head of cattle were followed up. Fortnightly controls of the parasitaemia and PCV were carried out during 8 weeks. The main trypanosome species was *T. vivax* (83.6%) followed by *T. congolense* (16.4%). In two villages, less than 25% of the control untreated cattle became positive indicating no need to use prophylactic treatment. These two villages were not further studied. Resistance to ISM was observed in 5 of the remaining 8 villages (Débé, Bendougou, Kangotenga, Mou and Laro) where the relative risk (control/treated hazard ratios) of becoming infected was lower than 2 i.e. between 0.89 (95% CI 0.43 – 2.74) and 1.75 (95% CI 0.57 – 5.37). In contrast, this study did not show evidence of resistance to DA in the surveyed villages with only 8.6% (n=93) of the cattle relapsing after treatment. Our results suggest that because of the low prevalence of multiple resistances in the area a meticulous use of the sanative pair system would constitute the best option to delay as much as possible the spread of chemoresistance till complete eradication of the disease by vector control operations.

Keywords: *Trypanosoma*; *vivax*; trypanocidal drugs; isometamidium; Burkina Faso; drug resistance; diminazene

## **Introduction**

The economy of Burkina Faso relies mainly on the rural sector which represents approximately 86% of the total 15 million inhabitants and contributes to about 40% to the 7.9 billion US\$ Gross Domestic Product (GDP) (MEDEV, 2002; World bank, 2009). Livestock production contributes for more than 10% to the GDP and is the second provider of foreign currency after the agricultural sector and before the mining sector (MEDEV, 2004; MRA, 2000). The Western and South-western parts of the country have a great potential for agricultural and livestock productions because of the relatively wet climate compared to the Northern part of the country. Unfortunately, the development of the livestock sector is there hindered by trypanosomosis. Currently, more than 63% of the Burkina Faso's cattle population, estimated at 2.5 million heads, lives in zones at risk of trypanosomosis (Kamuanga et al., 2001a; MRA, 2006).

The economic impact of animal trypanosomosis is difficult to assess. However, according to the Ministry for the Animal Resources of Burkina Faso, about 2.8 million of preventive or curative doses of trypanocides are used annually to support maintaining susceptible cattle breeds in the trypanosomosis endemic areas. The annual cost of trypanocides alone amounts to about 3.9 million US\$. According to the results of a socio-economic survey carried out in the pastoral zone of Yale and in the province of Kéné Dougou, the control of the trypanosomosis can induce a growth rate of 25% of the cattle population and can increase the daily dairy production up to ten times (Kamuanga et al., 2001b). In the province

of Kéné Dougou, the cost of the treatment of the disease per head of susceptible cattle was estimated at about 6.25 US\$ (Ouedraogo, 2002). Because of the problems associated with protecting tsetse cleared areas from re-invasion (Bauer et al., 1992; Bauer et al., 1995; Bauer et al., 1999; Cuisance et al., 1990), the use of trypanocidal drugs remains the main tool used by the livestock keepers to control the disease. Two drugs are mainly used: DA for treatment and ISM for prevention. Those drugs were marketed more than half a century ago and it is thus not astonishing that cases of drug resistance were reported in 18 countries of sub-Saharan Africa (Delespau et al., 2008) and more recently in Benin, Ghana and Togo (Réseau d'épidémiologie de la résistance aux trypanocides et aux acaricides en Afrique de l'Ouest – RESCAO, unpublished data). In Burkina Faso, chemoresistance was reported for the first time at the beginning of the 1980's in the province of Kéné Dougou (Authie, 1994; Pinder and Authie, 1984). The resistance to trypanocides was since reported in the other tsetse infested areas of Burkina Faso and especially the important cotton production zone (Clausen et al., 2010; Grace et al., 2009; McDermott et al., 2003). To date, no reliable or consistent data on trypanosome drug susceptibility is available for the Region of the Boucle du Mouhoun which is another important region for cattle breeding in Burkina Faso. The purpose of this study was thus (i) to evaluate the prevalence of the animal trypanosomosis in the area by means of a cross-sectional survey, (ii) to evaluate the level of the resistance to ISM in trypanosome by comparing the incidence of trypanosomal infections in treated and untreated herds during a period of 8 weeks (longitudinal survey), (iii) to evaluate the effect of the treatment against the infection by measure of the PCV in both groups and finally (iv) to determine the frequency of relapses after treatment with DA.

## Material and methods

### *Study area*

The Region of the Boucle du Mouhoun, is located in North-west Burkina Faso between longitudes -2.4° and -4.6° and latitudes 11.23° and 13.7°, covering a surface of about 34 500 km<sup>2</sup>. The area is subdivided into six provinces that are subdivided into 47 districts composed of a total of 1061 villages. The Region is located in the Sudano-Sahelian zone with an average annual rainfall ranging between 500 and 1400 mm (MEDEV, 2005). The area has a dense hydrographic network linked to the Mouhoun River (Figures 1a and 1b). The main socio-economic activities in the area are agriculture and livestock breeding. The Region hosts about 660.000 bovines and 1.450.000 small ruminants (MRA, 2006). The livestock breeding is extensive and making use of the natural pastures along the river edge and its basin. The cattle raised in the area are mainly zebus (*Bos indicus*), but taurines (*Bos taurus*) and cross-bred animals are also present. The area is infested with *Glossina palpalis gambiensis* and *G. tachinoides*, two riverine tsetse species, distributed unequally depending on the vegetation type and its degree of degradation by human activities (Guerrini et al., 2008). Their Index of Apparent Abundance (IAA) varies between 1 and 9.5 tsetse per trap and per day (Bouyer and Bengaly, 2006). The most frequent infection for both species is *T. vivax* (1.4%), followed by *T. congolense* (0.3%) and *T. brucei* (0.05%) (Kone et al., 2011). The parasitological and serological prevalence's of bovine trypanosomosis are 7% and 83% respectively (Bouyer and Bengaly, 2006).

### *Cross-sectional survey*

The cross-sectional survey was performed at the beginning of the dry season i.e. between September and December 2007. Among the 1061 villages of the 47 districts, one village was

randomly selected from each district. To increase the chances to get a suitable environment for the block testing i.e. where the risk of trypanosome infection is high enough, six additional villages were randomly selected from a list of 41 being located less than 5 km from the river or its main tributaries for a total of 53 villages. In each village 21 to 52 cattle were sampled. Animals were selected randomly from the herds present in each village with a maximum of 5 animals from any herd. A total of 2002 cattle were sampled. The buffy coat technique was used for the detection of trypanosomes (Murray et al., 1977). At least 40 fields were observed before considering a slide as negative. Blood samples that were positive were further processed as blood smears for trypanosome species identification. Giemsa-stained thin blood smears were examined under x 100 oil immersion objective lens (x1000 magnification).

#### *Longitudinal survey*

The longitudinal survey was conducted between February and May 2009 i.e. at the end of the dry season. Based on the outcome of the cross-sectional survey, the ten villages with the highest parasitological prevalence were selected. In each of the 10 villages, 74 to 100 cattle aged more than 1 year were identified and ear tagged. 96.2% were zebu cattle (*Bos indicus*), 3.6% crossbred and 0.2% taurine cattle (*Bos taurus*). The breed composition between treated and control groups was similar. The average age of the animals was 4.8 years with a standard deviation of 2.9 years.

Trypanocidal drug resistance was evaluated by means of treated and control sentinel herds as described by Eisler et al. (2000). Briefly, the selected cattle in each village (about hundred) were randomly divided in 2 equal groups, one being treated with ISM (Trypanidium® MERIAL) at a dose of 1mg/kg b.w. while the other remained untreated as

control. The animals were examined on day 0 (day of treatment with ISM), 14, 28, 42 and 56 for the presence of trypanosomes and PCV measurement to assess the efficacy of the treatment against the infection. Any positive animal was treated with DA (Trypadim® Merial) at 3,5mg/kg b.w..

The level of resistance to ISM was estimated by comparing the incidence of trypanosomal infections in the two groups (calculation of the relative risk, which is the ratio of the incidence in the control and the treated groups).

### *Statistical analysis*

The overall trypanosomal incidence data was analysed separately for *T. congolense* and *T. vivax* in Stata 10. Animals that were positive on the first day of observation were discarded from the analysis. Treatment was used as only explanatory variable to calculate the relative risk of infection in control animals compared to treated cattle. The hazard was assumed to be constant (exponential distribution) in all survival analysis for ease of interpretation.

Variations of treatment efficiency in the different study sites were further evaluated for *T. vivax* infections. Here, site, treatment and the interaction between the two were used as explanatory variables. Sites with low number of events were excluded from this part of the analysis. For each site, the logarithm of the relative risk of treated animals and its 95% confidence interval were calculated by summing the model coefficients of the treatments and site/treatment interaction explanatory variables (linear combination of estimators in Stata 10). Actual relative risks and 95% confidence intervals were derived by calculating the exponential of these values. The monthly hazard and its 95% confidence interval were

calculated using the exponential of the linear predictions and considering that a month is made of 28 days.

PCV values of *T. vivax* infected animals recorded 8 to 56 days after treatment in the ISM and control groups were compared in a linear regression using the treatment as only explanatory variable. PCV values were arcsine transformed to ensure normality of the distribution of the response variable (Osborne, 2002). The homoscedasticity of the model and the normality of the residuals' distribution were checked. The final model used the individual animals as random effects to account for repeated measures. A possible seasonal effect was not considered as the longitudinal survey was completely conducted within the 4 last months of the dry season.

## **Results**

### *Cross sectional survey*

The results are summarized in table 1. Trypanosomosis was observed in 13 villages.

Infections were mainly (88.5%) caused by *T. vivax* whereas *T. congolense* was found in only 2 villages. No mixed infections were recorded. The prevalence reached 7.4%, 9.6% and 16.1% at Débé, Kangotenga and Soukoura respectively.

### *Longitudinal survey*

The longitudinal study was conducted in the 10 villages with the highest prevalence (ranging from 2.1 to 16.1%). Among the 978 sentinel cattle selected from 70 different herds, 492 were treated with ISM and 486 served as a control. During the block treatment study a total of 250 new trypanosomal infections were detected. 83.6% (n=209) and 16.4% (n=41) of the



infections were due to *T. vivax* and *T. congolense* respectively. One animal only presented a mixed infection.

Considering the infections with *T. congolense*, the monthly (28 days) hazard of *T. congolense* infections in control cattle was 3.2% (95% CI: 2.2-4.5%). The overall relative risk (control/treated hazard ratios) of *T. congolense* infections was 4.3 (95% CI: 1.9-10). The analysis of the relative risk at site level was not performed in regard of the low number of new infections with *T. congolense*.

The monthly hazard of *T. vivax* infections in control cattle was 12% (95% CI 10-14%) and the overall relative risk of *T. vivax* infections for the 10 villages was 1.8 (95% CI: 1.3-2.5).

Because the number of new infections was low in cattle sampled at St Michel and Soukoura with one and no new infection in the ISM-treated groups and 4 and 2 new infections in control groups respectively, these two villages were excluded from the analysis of the relative risk at site level.

The total number of new *T. vivax* infections in each of the 8 remaining villages and in each of the experimental groups during the 8 weeks observation period is presented in table 2. The monthly hazard of new infections during the 8 weeks observation period in the control groups varied between 24.3% and 59.5%. The relative risk ranged from 0.9 in Laro to 3 in Déré and varied significantly between sites. However, the interactions between sites and treatment were not significant (i.e. the effect of treatment did not significantly vary in the different sites;  $p=0.47$ ) (Table 2). Five villages (Débé, Bendougou, Kangotenga, Laro and Mou) presented a relative risk lower than two strongly suggesting ISM resistance (Figure 2 and Table 2).

The PCV values of cattle infected with *T. congolense* were not further analysed because of the low number of observations. Interestingly, ISM-treated animals developing a parasitaemia (*T. vivax*) presented a mean PCV of 0.30 (95% CI 0.28-0.31) significantly higher than the infected animals from the control group with a value of 0.26 (95% CI 0.25-0.28).

During the follow-up, 93 animals of the control group needed to be treated with DA. Within 14 days post treatment, 8.6% only of the treated animals relapsed (Table 3) suggesting no acute problem of resistance to DA.

## **Discussion**

The first reports of trypanocidal drug resistance in Burkina Faso date from the early 1980's in the province of Kénédougou (Authie, 1994; Pinder and Authie, 1984) and were focused on *T. congolense*. Worrying multi-drug resistance to ISM, DA and ethidium bromide was then observed by Clausen et al. (1992). Further studies confirmed those observations (Clausen et al., 2010; McDermott et al., 2003; Talaki et al., 2007). When considering *T. vivax*, albeit case reports of resistance to ISM or DA has been described in East and West Africa (Gray and Roberts, 1971; Kupper and Wolters, 1983; Mwambu and Mayende, 1971; Schonefeld et al., 1987), the information is scarcer compared to the data available for *T. congolense*. The reason for the delay in the reporting of trypanocidal drug resistance in *T. vivax* is largely due to (i) the preponderance of *T. congolense* infections in large parts of tsetse-infested Africa, (ii) the difficulties associated with the isolation of *T. vivax* in laboratory animals and (iii) the absence of any molecular diagnostic tool for this species.

Our observations confirm thus the strong suspicion of resistance to ISM in the Region of the Boucle du Mouhoun. Indeed, based on the criteria used by Eisler et al. (2000), a relative risk

lower than two strongly suggest resistance to ISM. No resistance was observed in three villages (Déré, Nokuy and Boromissi). This cannot be attributed to a poor challenge since the incidence of the control herds in these villages were among the highest (figure 2).

Resistance was observed in 5 out of the 8 villages included in the study (i.e. Laro, Bendougou, Débé, Kangotenga and Mou), though the upper limit of the 95% confidence interval was always >2.5. Nevertheless, no difference was observed in the level of resistance between the different sites, i.e. the relative risks between the 5 sites where ISM resistance was detected were not statistically different. It should here be emphasized that ISM was used as recommended by Eisler et al. (2000) at a dose of 1mg/kg b.w. rather than the dosage that is commonly used in the field by veterinary technicians, i.e. 0.5mg/kg b.w.. The low overall relative risk of *T. vivax* infections (1.8 with 95% CI: 1.3-2.5) with this dosage of ISM twice higher than commonly used is a supplementary argument in favour of the existence of ISM resistance. Reasons for this widespread distribution of resistance are attributed to a range of factors such as the long-term use of the same molecules, the misuse of the drugs and the often low quality of drugs available on the local markets (Geerts et al., 2001). Over the 2.8 million doses of trypanocides are used annually in Burkina Faso (MRA, 2006) of which only 23% are officially imported. Recent studies on the quality of the trypanocides DA and ISM sold in sub-Saharan Africa, showed that a great majority of these products do not respect the standards established by the original producers (Schad et al., 2008; Tettey et al., 2002). Recently, the Ministry of Animal Resources of Burkina Faso in collaboration with FAO financed an investigation to determine the quality of the veterinary drugs on the local markets. The study showed that about half of the trypanocides were not in conformity with the quality requirements (Têko, personal communication). According to

the official report of the MRA (2006), the annual use of trypanocides in the area of the Boucle du Mouhoun decreased from 1,054,004 doses to 77,910 doses between 2003 and 2006. This drastic drop in the official sales figures reflects the existence of parallel providers of veterinary drugs including trypanocides.

Notwithstanding the observed resistance in *T. vivax* and the resulting relapses in treated animals, treatment with ISM still seems to have a beneficial effect on the condition of the animals. This is reflected by the higher PCV values of cattle infected by ISM resistant trypanosomes and treated with ISM compared to untreated animals of the control herds. The fact that animals from the control herds were followed up every two weeks and treated with DA when positive obviously decreased the difference between ISM-treated and non-treated cattle. In uncontrolled field conditions, the beneficial effects of treating with ISM would thus be even more apparent. This phenomenon was already observed for *T. congolense* in an experimental model where cattle were inoculated with ISM-resistant trypanosomes. The impact of the infection on the PCV was not very pronounced with an average PCV reduction 8 to 14 weeks after treatment of only 5.9% (95% CI: 4.5–7.3) (Delespaux et al., 2010).

## **Conclusion**

Chemoresistance in the region of the Boucle du Mouhoun seems to remain manageable as no evidence of multi-resistance was found. A rational use of the sanative pair technique is certainly recommended to maintain the situation at controllable level. The concept of the sanative pair recommends the use of two trypanocides (e.g. DA and ISM) which are chemically unrelated and, therefore, are unlikely to induce cross-resistance. The first pair is

used until resistant strains of trypanosomes appear and then the second is substituted and used until the resistant strains have vanished from cattle and tsetse (Whiteside, 1962). However, in field conditions the choice of the drug is rather determined by the availability and price than by strategic options. Furthermore, as demonstrated in this study, animals infected with drug resistant trypanosomes and treated with ISM will have a better health condition and productivity which will encourage farmers to continue treating. A tsetse eradication program was initiated by the PATTEC in the area that will probably improve the livelihood of the farmers in the near future. However, as *T. vivax* can be mechanically transmitted, trypanocidal drugs have still to be used strategically till complete eradication of the disease.

### **Acknowledgement**

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**Table 1: Prevalence of bovine trypanosomosis in various villages located in the Boucle du Mouhoun Region of Burkina Faso**

Village	Number sampled	Number positive	Prevalence (%)	<i>Trypanosoma</i> spp.	
				<i>T. vivax</i>	<i>T. congolense</i>
Laro	47	1	2.1	1	0
Mou	23	1	4.3	1	0
Boromissi	25	1	4.0	1	0
St Michel	44	2	4.6	2	0
Déré	51	2	3.9	2	0
Bendougou	21	1	4.8	1	0
Bamakoro	46	1	2.2	1	0
Nokuy	36	2	5.6	2	0
Saorokuy	50	1	2	2	0
Soukoura	31	5	16.1	3	2
Zelassé	44	1	2.3	1	0
Kangotenga	52	5	9.6	4	1
Débé	27	2	7.4	2	0

**Table 2: Predicted monthly hazard of *T. vivax* infection in ISM-treated and control groups and relative risk (control/treated hazard ratios) for each of the study sites.**

Villages	Group	Number of animals	Number of <i>T. vivax</i> infections	Monthly hazard (%)	Relative risk	95% confidence interval
Débé	Treated	50	10	9.8	1.09	0.43-2.74
	Control	49	12	10.7		
Laro	Treated	37	8	7.6	0.89	0.30-2.65
	Control	37	9	6.8		
Déré	Treated	43	8	9.1	3.04	1.33-6.97
	Control	39	17	25.3		
Bendougou	Treated	50	9	5.1	1.75	0.57-5.37
	Control	50	21	8.7		
Nokuy	Treated	45	9	7.1	2.17	0.80-5.86
	Control	43	21	14.9		
Kangotenga	Treated	45	11	8.5	1.15	0.44-2.98
	Control	44	11	9.7		
Mou	Treated	40	15	16.4	1.67	0.84-3.34
	Control	42	25	26.0		
Boromissi	Treated	48	7	5.2	2.70	0.95-7.68
	Control	49	16	13.6		

**Table 3: Number of animals belonging to the control groups relapsing after treatment with diminazene aceturate (3.5mg/kg b.w.) within 14 days post treatment.**

Village	Number treated	Number relapsed (%)
Déré	23	2 (8.7)
Nokuy	34	2 (5.9)
Kangotenga	19	3 (15.8)
Laro	17	1 (.9)

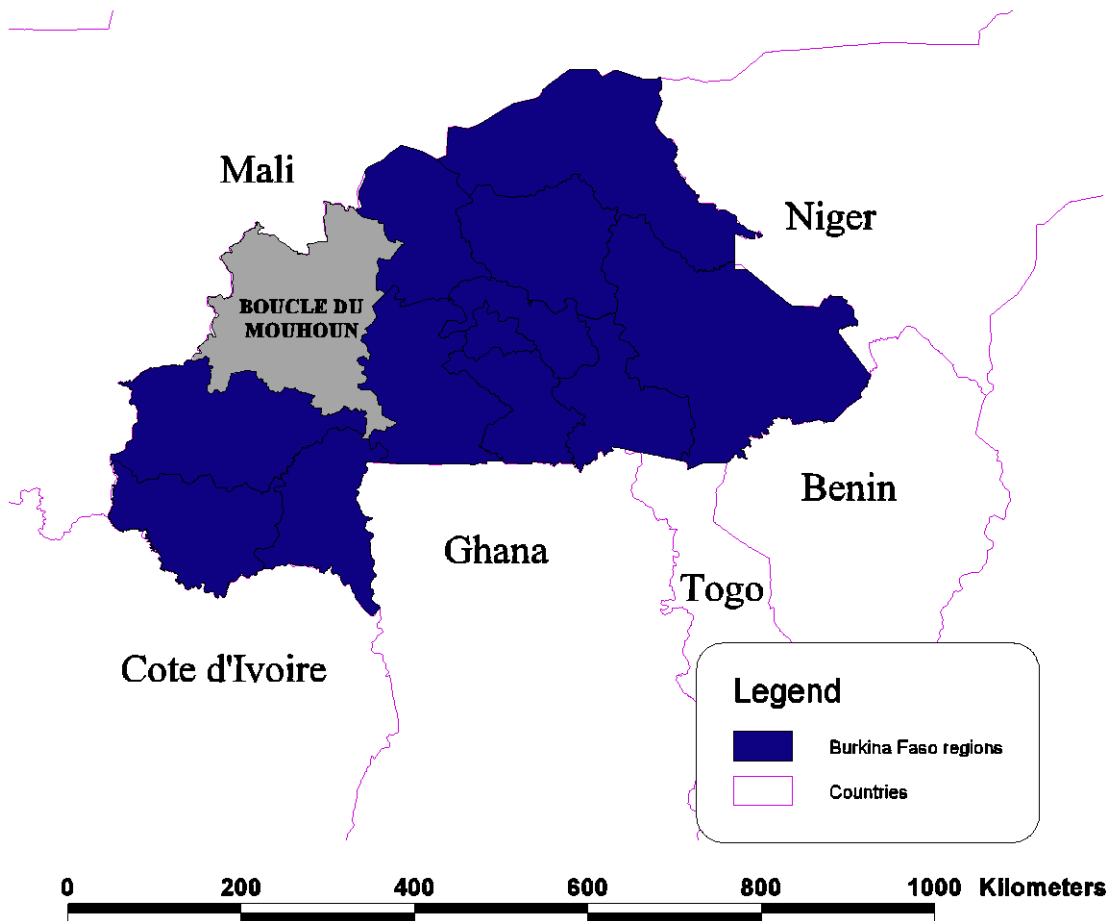


## Figure captions

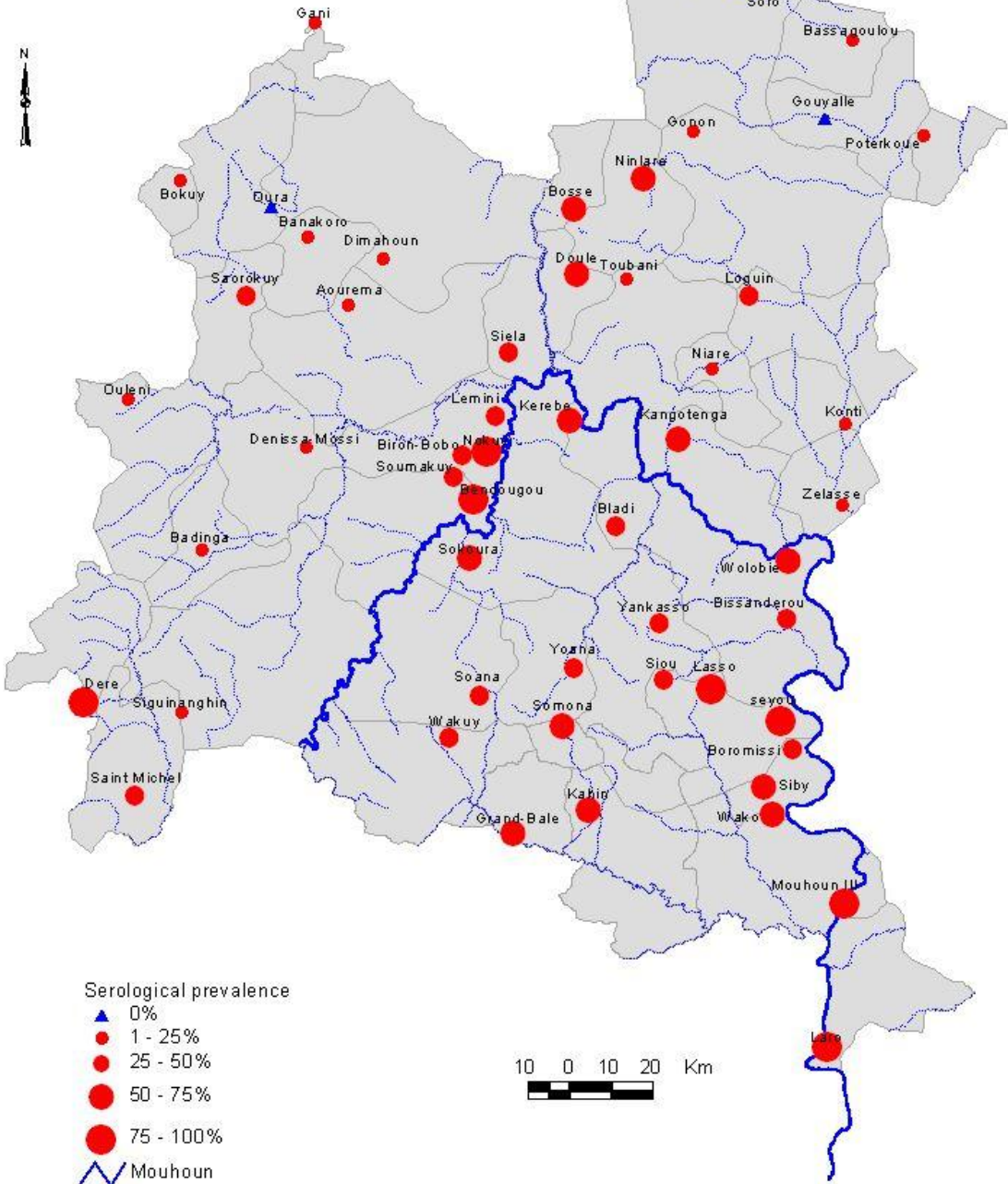
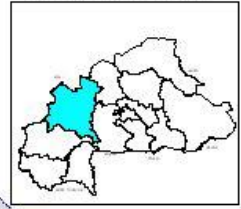
Figure 1a Localization of the study area

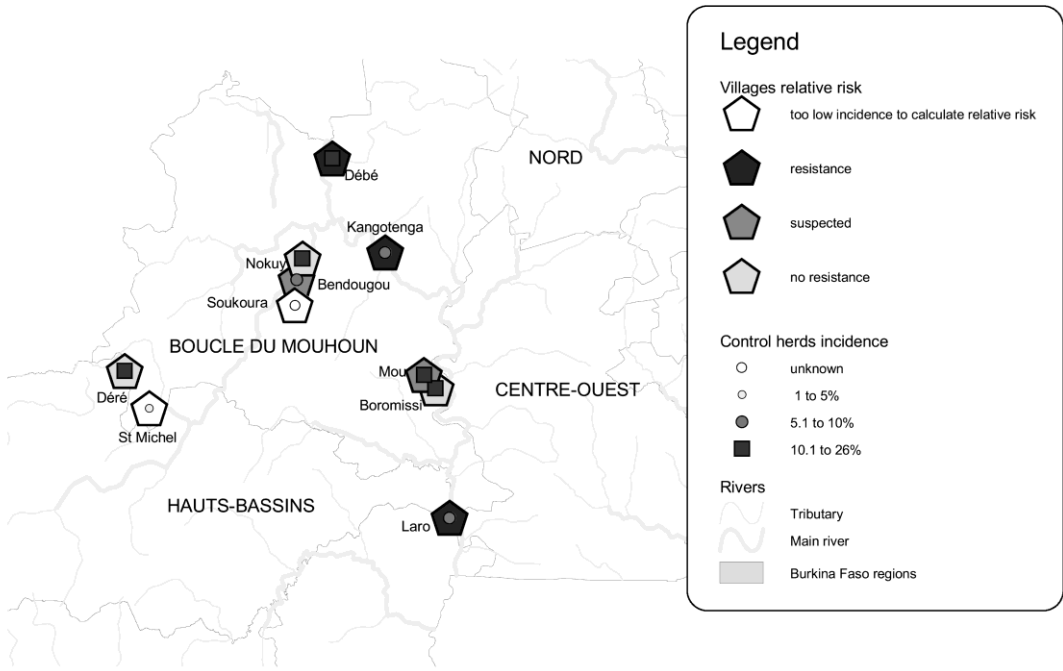
Figure 1b: Localization of the study area, cross-sectional study sites and villages where the block treatments were conducted.

Figure 2 Villages relative risk and control herd incidence



PLAN DE SITUATION





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