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Is trypanocidal drug resistance a threat for livestock health and production in endemic areas? Food for thoughts from Sahelian goats infected by *Trypanosoma vivax* in Bobo Dioulasso (Burkina Faso)

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ABSTRACT

Trypanocidal drug resistance is unanimously recognized as a threat for livestock production in regions where the prevalence of trypanosomosis is high. To assess the impact of the disease and the effect of drug resistance on the health of small ruminants, twelve *Trypanosoma vivax* isolates collected in 6 villages in the vicinity of Bobo Dioulasso (Burkina Faso) were injected into 12 groups of 5 Sahelian goats, two being treated with 3.5 mg/kg body weight diminazene aceturate (DA), two with 0.5 mg/kg body weight isometamidium chloride (ISM) and one left untreated as control. A monitoring was performed every 5 days for 100 days to evaluate the parasitaemia by buffy coat examination, the hematocrit and the body weight. Among the 12 groups, 6 were additionally monitored using a trypanosome specific 18S-PCR–RFLP every 5 days from day 30 to day 100 to verify the complete clearance of the parasites from the blood of the hosts. In six groups of goats, trypanosomes disappeared completely after treatment, five groups showed relapses in at least one goat treated with ISM and one group showed relapses in one goat treated with DA and one with ISM. For the 6 groups that were screened both using microscopic examination and trypanosome specific 18S-PCR–RFLP, the following results were observed: for the groups treated with DA, no relapses by microscopic examination and 83.3% (10/12) using the 18S-PCR–RFLP. For the groups treated with ISM, 25% (3/12) relapses by microscopic examination and 83.3% with the 18S-PCR–RFLP (10/12). The evolution of the PCV and the weight during the observation period from relapsing (either by microscopical examination or by 18S-PCR–RFLP diagnosis) and non relapsing animals were compared. The relative average PCV in goats that relapsed microscopically, decreased significantly more than in non-relapsing goats. This difference was not significant when relapses were detected using the trypanosome specific 18S-PCR–RFLP. This indicates that only the animals with the highest parasitaemia suffered from the infection. Relapses after treatment where the host controls the parasitaemia to a level below the sensitivity of the microscopical examination do not affect body weight nor PCV.

1. Introduction

Chemotherapy and chemoprophylaxis of trypanosomosis in cattle, sheep and goats continue, in most endemic and epidemic areas, to heavily rely upon the administration

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of the salts of three compounds: ethidium, isometamidium (ISM) and diminazene (DA). Those three drugs have been used for more than 50 years and resistance to these compounds has been reported in 18 African countries (Delespaux et al., 2008) and more recently in Benin, Ghana and Togo (Réseau d'épidémiosurveillance de la résistance aux trypanocides et aux acaricides en Afrique de l'Ouest – RESCAO, unpublished data). More countries are most probably affected by this trypanocide-resistance problem but the logistics that are required for standardized testing (block treatment, in vivo and in vitro tests) are logistically heavy leading to a rather scarce reporting of the drug resistance occurrence. This situation is even worse for the detection and reporting of drug resistance in *Trypanosoma vivax* as this parasite species is not able to multiply in the experimental rodents. As a consequence, experimental testing makes it necessary to inoculate and treat calves or goats, which represent an expensive and labor intensive exercise. Trypanocidal drug resistance in Burkina Faso has been reported for *Trypanosoma congolense* by several authors (Clausen et al., 1992; McDermott et al., 2003; Knoppe et al., 2006) but no data is available for *T. vivax*.

The diagnosis of relapses after treatment is mainly done by microscopic examination, which seriously underestimate the effective relapsing rate and more seldom by the existing molecular tools (Gall et al., 2004). This noticeable difference in sensitivity between the two techniques for the detection of relapses after treatment was already described in a mice model (Chitanga et al., 2011). Furthermore, it was showed that the fluctuating low parasitaemia following the treatment of cattle with ISM after inoculation with ISM-resistant strains of *T. congolense* (Delespaux et al., 2010) had also limited impact on the PCV of the parasitaemic animals. From those observations, the hypothesis was made that latent low parasitaemia infections caused by drug resistant trypanosomes were actually compatible with the survival and an acceptable productivity of their host.

The objectives of this study were thus (i) to evaluate the sensitivity of local *T. vivax* strains to the two main trypanocidal drugs used in the field i.e. ISM and DA, and (ii) to compare the effects of the treatments on the hematocrit and the body weight in animals relapsing after diagnosis by microscopical examination or diagnosis with a trypanosome specific 18S-PCR and in animals completely cleared from parasites.

2. Materials and methods

2.1. Experimental animals

60 female goats aged between 1 and 3 years, belonging to the Sahelian breed were selected from the North-Eastern region of Burkina Faso (Dori), an area free of tsetse flies (Bengaly et al., 2001; Courtin et al., 2010). The goats were housed in fly-proof facilities. Before the experiment, they were treated with DA at the blanking dose of 7 mg/kg B.W. and dewormed with oxfenbendazole (4.5 mg/kg B.W.). Due to endemic pasteurellosis in the area, the animals were vaccinated against this disease and then quarantined for a month. They were fed with fresh or dry straw, supplemented with cotton seed and watered ad libitum with tap water during the quarantine and the experiment. A trypanosome specific 18S-PCR–RFLP (Geysen et al., 2003; Delespaux et al., 2003) was performed on buffy coats sampled from each goat to verify the absence of trypanosomes at the moment of inoculation.

Table 1
GIS coordinates of the sampling sites.

Village	*X* coordinate	*Y* coordinate
Dafinso	−4.22493	11.28616
Débé	−4.47417	12.03837
Dèrè	−4.28566	11.15875
Kadomba	−4.00108	11.50257
Kangotenga	−3.16267	12.60502

2.2. Trypanosome isolates

The isolates were sampled in 6 villages located in the vicinity of Bobo Dioulasso, Burkina Faso (see GIS coordinates in Table 1). Species diagnosis was performed using a trypanosome specific 18S-PCR–RFLP (Geysen et al., 2003; Delespaux et al., 2003). Trypanosome-infected blood samples were cryopreserved in a 5% final concentration of DMSO and stored in liquid nitrogen until characterization. Details on those strains are provided in Table 2.

2.3. Experimental infection

Each of the six *T. vivax* cryostabilates was thawed at 37 °C and reactivated through a passage in a goat. Parasitaemia was monitored by microscopical examination of the buffy coat as described by Murray et al. (1977). At the first peak of the parasitaemia, blood was collected and diluted with PBS/Glucose (5%) to a final concentration of 10^5 trypanosomes/ml.

For each isolate, five goats were ear-tagged and inoculated intravenously with 10^5 reactivated trypanosomes. At the first observation of parasites in the blood of one of the five animals of a group, two were treated with 3.5 mg/kg B.W. DA, two with 0.5 mg/kg B.W. ISM and one left untreated as control. A dose of 7 mg/kg DA was used for animals showing a life-threatening PCV (below 15%) during the observation period. For these animals, observations were stopped at that moment.

2.4. Monitoring

After inoculation, the goats were parasitologically monitored every 5 days by sampling blood from the jugular vein with heparinized Vacutainer® tubes (BD Medical). From those tubes, blood was collected into glass capillary tubes without anti-clotting agent and stopped at an extremity with Cristaseal® (Hawxley). After centrifugation at 9000 rpm for 5 min, the values of the hematocrit were recorded. Buffy coats were examined using a microscope (×400) for the detection of trypanosomes. A slide was considered as negative if no trypanosome was observed in 50 fields. For each animal, one buffy coat was placed on a filter paper (Whatman N°4, Whatman®), dried protected from UV light, stored in individual envelopes that were placed in plastic bags containing silica gels and conserved at −20 °C

Table 2
Trypanosoma vivax isolates used in the in vivo goat sensitivity test.

Groupe	Code[a]	Host	Village	Country
1	KAD 41 Tv/F, 10/11/10	Bovin	Kadomba	Burkina Faso
2	D 39 Tv/F, 13/11/10	Bovin	Dafinso	Burkina Faso
3	D 42 Tv/F, 13/11/10	Bovin	Dafinso	Burkina Faso
4	K 56 Tv/F, 12/11/10	Bovin	Koumbia	Burkina Faso
5	K 30 Tv/F, 8/11/10	Bovin	Koumbia	Burkina Faso
6	K 4 Tv/F, 8/11/10	Bovin	Koumbia	Burkina Faso
7	D 32 Tv/F, 13/11/10	Bovin	Dafinso	Burkina Faso
8	K 28 Tv/F, 8/11/10	Bovin	Koumbia	Burkina Faso
9	DE 35 Tv/Ch.T3, 02/11/09	Caprin	Dèrè	Burkina Faso
10	DEB 53 Tv/F, 09/04/09	Bovin	Débé	Burkina Faso
11	KA 1 Tv/F, 08/04/09	Bovin	Kangotenga	Burkina Faso
12	DE 57 Tv/F, 03/04/09	Bovin	Dèrè	Burkina Faso

[a] All strains collected for the Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES).

for subsequent molecular analysis with the trypanosome specific 18S-PCR–RFLP (Geysen et al., 2003; Delespaux et al., 2003). The RFLP step necessary for the trypanosome species determination was conserved, even if the species that was inoculated was known, to confirm the specificity of the PCR reaction. The DNA stored on the filter papers was extracted using the routine PBS-Saponin technique (de Almeida et al., 1997). The parasitaemia, the hematocrit and the body weight of the goats were controlled every 5 days after the treatment with the trypanocides for 100 days.

2.5. Statistical analysis

PCV data were analyzed using cross-sectional time-series linear regressions in Stata 10, separately for DA and ISM treatments. Categorical explanatory variables were the goat status (no relapse, relapse at microscopy or relapse at PCR), the period of observation (<15, 15–44 or ≥45 days post-infection) and the interactions between the two. Individual goats were considered as random effects. Finally, relative PCV differences between periods (using period 1 as denominator) were calculated using the non-linear combination of estimators (delta method). The difference of relative differences was calculated using the following equations:

$$\mathrm{DRD_{PCV}} = \left(\frac{\mathrm{PCV_{Rx}} - \mathrm{PCV_{R1}}}{\mathrm{PCV_{R1}}}\right) - \left(\frac{\mathrm{PCV_{NRx}} - \mathrm{PCV_{NR1}}}{\mathrm{PCV_{NR1}}}\right)$$

$$\mathrm{DRD_{w}} = \left(\frac{W_{RX} - W_{R1}}{W_{R1}}\right) - \left(\frac{W_{NRX} - W_{NR1}}{W_{NR1}}\right)$$

where DRD is the difference of relative differences; PCV the packed cell volume; *W* the weight; R the non-relapsing goats; 1 the Period 1 or day 1 and *x* is the period 2 or 3 or day 2, 3 or 4

Weight data were analyzed in similar models, except that explanatory variables were the goat status, time of observation (days 0, 30, 60 and 90 post-infection) and the interactions between the two.

3. Results

3.1. Health condition

Control and treated goats presenting severe clinical signs or a PCV below 15 were treated with 7 mg/kg B.W. which caused in all cases an increase of the PCV back to physiological values or at least higher than 20%. Nine goats from 6 ISM groups (1, 2, 5, 9, 10 and 11) and the 2 goats from DA group 2 had to be treated with a high dose of DA to rescue them from certain death. This corresponds to all the relapses diagnosed by microscopic examination (see Table 3).

3.2. Sensitivity tests of the T. vivax strains

Among the 12 groups of four goats (control not included) that were screened by microscopical examination, six were completely cured after treatment, 5 showed relapses in at least one goat treated with ISM and 1 showed relapses in all treated goats. The data are summarized in Table 3.

For the 6 groups that were additionally screened with the trypanosome specific 18S-PCR–RFLP, the following results were observed: for the groups treated with DA, no relapses by microscopic examination and 83.3% (10/12) using the 18S-PCR–RFLP. For the groups treated with ISM,

Table 3
Results of the microscopic examination of the 60 goats.

	DA1	DA2	ISM1	ISM2	C
Group 1	–		+	–	+
Group 2	+	+	+	+	+
Group 3	–	–	–	–	+
Group 4	–	–	–	–	+
Group 5	–	–	+	–	+
Group 6	–	–	–	–	+
Group 7	–	–	–	–	+
Group 8	–	–	–	–	+
Group 9	–	–	–	+	+
Group 10	–	–	+	+	+
Group 11	–	–	+	+	+
Group 12	–	–	–	–	+

With + as animal found microscopically positive during the examination period, DA*x* goat treated with DA, ISM*x* goat treated with ISM and C as control.

Table 4
Comparison of the results of the microscopic examination and the 18S-PCR for 24 goats.

	Microscope				PCR			
	DA1	DA2	ISM1	ISM2	DA1	DA2	ISM1	ISM2
Group 1	N	N	6*x*	N	3*x*	3*x*	6*x*	4*x*
Group 3	N	N	N	N	4*x*	N	3*x*	N
Group 4	N	N	N	N	2*x*	3*x*	1*x*	N
Group 5	N	N	N	8*x*	3*x*	3*x*	3*x*	8*x*
Group 6	N	N	N	N	3*x*	5*x*	3*x*	3*x*
Group 9	N	N	N	8*x*	2*x*	N	1*x*	8*x*
Total relapses	0/6	0/6	1/6	2/6	6/6	4/6	6/6	4/6

With N as negative, XX as number of times that an animal was observed positive during the 100 days observation period (every 5 days), DA*x* goat treated with DA and ISM*x* goat treated with ISM.

25% (3/12) relapses by microscopic examination and 83.3% (10/12) with the 18S-PCR. The data are summarized in Table 4.

3.3. PCV and weight evolution of DA relapsing goats compared to DA non-relapsing goats

The relative average PCV in goats that relapsed microscopically, decreased significantly more than in non-relapsing goats. The difference between the relative average PCV reduction between period 1 (day 0–14) and period 2 (day 15–44) in relapsing and non-relapsing goats was estimated at 24.9% (95% CI 17.9–31.9%). This difference was not significant when relapses were detected using the trypanosome specific 18S-PCR–RFLP and this when comparing period 1 to period 2 or period 1 to period 3 (≥45 days). No difference was observed in the weight evolution between relapsing or non relapsing goats and this for all comparisons in time i.e. time 1 versus 2, 1 versus 3 and 1 versus 4. The results for the weight evolution are summarized in Table 5.

3.4. PCV and weight evolution of ISM relapsing goats compared to ISM non-relapsing goats

The relative average PCV in goats that relapsed microscopically, decreased significantly more than in non-relapsing goats. The difference between the relative average PCV reduction between period 1 and period 2 in relapsing and non-relapsing goats was estimated at 10.1% (95% CI 15.4–4.8%). This difference was also significant when relapses were detected using the trypanosome specific 18S-PCR–RFLP (difference of average PCV reduction between period 1 and period 2 in relapsing and non-relapsing goats: 8.4–95% CI 14.4–2.4% and difference of average PCV reduction between period 1 and period 3: 12.5–95% CI 17.7–7.2%). The goats that relapsed microscopically presented significantly higher relative weight losses for all the three recording times compared to day 0. Relapses at 18S-PCR–RFLP did not present significantly higher relative weight loss than non-relapsing goats and this for the three recording times. The results are summarized in Table 6.

4. Discussion

Animal health and production in zones that are endemic for trypanosomosis has always be challenging from an economic perspective. Decreasing the impact of the disease on the health status of their animals is often the only possible strategy for farmers with scarce financial resources. Some farmers act strategically by preferring trypanotolerant animals or even by using the zero-grazing system or confining animals in net-protected units (Bauer et al., 2006). Yet, in most cases, farmers rely on trypanocidal drugs that are administered with different levels of know-how (Grace et al., 2009) but also often without any parasitological diagnosis or even clinical examination (Van den Bossche et al., 2000). The sole trypanocides available for animal use have been marketed for more than half a century. Considering the nearly blind and routine administration of those products, it is not astonishing that drug resistance prevails in many regions of Africa (Delespaux et al., 2008). Even if constructive initiatives were implemented for promoting a rational use of trypanocides (Liebenehm et al., 2011), the prevailing situation is described by many authors as very alarming (Chaka and Abebe, 2003; Mamoudou et al., 2008). However, our experimental observations in a mice

Table 5
Weight volutions of relapsing goats compared to non-relapsing after treatment with DA with probabilities and 95% confidence intervals.

Comparison	Observed reduction	Probability	95% up	95% low
Micro 1–2	3.73%	0.44	−5.70%	13.16%
Micro 1–3	−6.05%	0.21	−15.51%	3.40%
Micro 1–4	−7.58%	0.12	−17.08%	1.91%
Pcr 1–2	2.27%	0.46	−3.80%	8.33%
Pcr 1–3	1.97%	0.53	−4.11%	8.05%
Pcr 1–4	0.75%	0.81	−5.33%	6.83%

Difference of the relative weight reduction (using weight at time 1 as denominator) in relapsing and non-relapsing goats. With Micro *x*–*x* as the comparison of the relative weight loss of animals relapsing microscopically between period *x* and *x*; Pcr *x*–*x* as comparison of the relative weight loss of animals relapsing when examined by Pcr between period *x* and *x*.

Table 6
Weight evolutions of relapsing goats compared to non-relapsing after treatment with ISM with probabilities and 95% confidence intervals.

Comparison	Observed reduction	Probability	95% up	95% low
Micro 1–2	−9.38%	0.01	−16.35%	−2.40%
Micro 1–3	−14.96%	0.00	−22.00%	−7.92%
Micro 1–4	−17.14%	0.00	−24.22%	−10.06%
Pcr 1–2	−2.43%	0.53	−10.03%	5.17%
Pcr 1–3	−2.98%	0.45	−10.67%	4.70%
Pcr 1–4	−2.60%	0.51	−10.37%	5.17%

Difference of the relative weight reduction (using weight at time 1 as denominator) in relapsing and non-relapsing goats. With Micro *x*–*x* as the comparison of the relative weight loss of animals relapsing microscopically between period *x* and *x*; Pcr *x*–*x* as comparison of the relative weight loss of animals relapsing when examined by Pcr between period *x* and *x*.

model where ISM-resistant trypanosomes were inoculated into ISM-treated and untreated animals suggests that even when trypanocidal drug resistance is evident, it might be relevant to treat the infected animals. Indeed, when cattle were experimentally inoculated with ISM resistant *T. congolense* strains and treated with ISM at the first observation of parasites in the blood, the effects of the trypanosome infection on the PCV remained very discrete (average PCV reduction 8–14 weeks after treatment: 5.9%; 95% CI: 4.5–7.3) (Delespaux et al., 2010).

From this study on the sensitivity of different *T. vivax* isolates to the two main trypanocidal drugs used in the field i.e. ISM and DA, we can conclude that drug resistance for these parasites is present but still manageable in the region around Bobo Dioulasso as only 1/12 isolate was found resistant to both drugs and 5/12 resistant to ISM only allowing in most instances the use of the sanative pair (use of DA to eliminate ISM-resistant strains and vice versa). The multi-resistant isolate albeit highly virulent was cleared microscopically with a double dose of DA (7 mg/kg).

The presence of drug resistance in *T. vivax* in Burkina Faso being confirmed, the second aim of this study was to evaluate the effects of the treatment in animals inoculated and treated at the first observation of parasites and to compare the evolution of the weight and PCV in (i) microscopically relapsing animals, (ii) animals relapsing when diagnosed by the trypanosome specific 18S-PCR–RFLP and finally (iii) animals completely cured and this in controlled conditions but the closest possible to field conditions.

As reasonably expected, the relative PCV reductions were significantly higher in animals relapsing microscopically i.e. in animals showing the highest parasitaemia. For the relapsing animals diagnosed using the trypanosome specific 18S-PCR–RFLP i.e. animals with the lowest parasitaemia, the fact that the relative PCV reductions were only significant after treatment with ISM, suggests a higher degree of ISM-resistance compared to DA. A lower resistance against DA would allow the host immunity together with the temporary toxic effect of the drug to keep the parasite under control. This might not be the case with ISM for which higher resistance would not allow animals to control the parasite as efficiently as with DA in spite of the fact that the elimination of ISM from the host is far slower (approximately 2 months compared to two weeks for DA). Yet, the absence of significant effect in DA treated animals could be attributed to the low number of relapses with DA.

Interestingly, the animals showing a relapse with a high parasitaemia (microscopic diagnosis) presented the highest relative PCV reductions (24.9% and 10.1% after treatment with DA and ISM respectively). It can be reasonably assumed that, as it was observed in *T. congolense* (Masumu et al., 2006), the virulence of different strains of *T. vivax* might vary and, among the drug resistant parasites, the most virulent only will cause visible deleterious effects on the health of the relapsing animals. Furthermore, self-cure appears to be relatively common in *T. vivax* infections (Gardiner, 1989) and it was observed that animals that recovered spontaneously from an acute infection developed chronic and asymptomatic infections (Batista et al., 2009). The treatment with trypanocide combined with the immune system of the host could increase this recovery rate by allowing a better control of the parasite and resulting in very low oscillating parasitaemia with no or little impact on the host's health.

As hypothesized above for PCV, the significantly higher weight losses in ISM relapsing animals (diagnosed using microscopy i.e. with the highest parasitaemia) might be explained by a higher resistance against this particular drug compared to DA.

5. Conclusions

This study showed that trypanocidal drug resistance had no significant impact on the PCV and body weight losses of goats infected by *T. vivax* except for a few highly virulent strains. This conclusion contradicts the general believe that the development of resistance against trypanocides would leave farmers helpless in trypanosome infested areas.

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References

Batista, J.S., Oliveira, A.F., Rodrigues, C.M.F., Damasceno, C.A.R., Oliveira, I.R.S., Alves, H.M., Paiva, E.S., Brito, P.D., Medeiros, J.M.F., Rodrigues,

A.C., Teixeira, M.M.G., 2009. Infection by *Trypanosoma vivax* in goats and sheep in the Brazilian semiarid region From acute disease outbreak to chronic cryptic infection. Vet. Parasitol. 165, 131–135.

Bauer, B., Gitau, D., Oloo, F.P., Karanja, S.M., 2006. Evaluation of a preliminary title to protect zero-grazed dairy cattle with insecticide-treated mosquito netting in Western Kenya. Trop. Anim. Health Prod. 38, 29–34.

Bengaly, Z., Ganaba, R., Sidibe, I., Desquesnes, M., 2001. Trypanosomose animale chez les bovins dans la zone Sud-soudanienne du Burkina Faso. Rev. Elev. Méd. vét. Pays trop. 54, 221–224.

Chaka, H., Abebe, G., 2003. Drug resistant trypanosomes: a threat to cattle production in the Southwest of Ethiopia. Rev. Elev. Méd. vét. Pays trop. 56, 33–36.

Chitanga, S., Marcotty, T., Namangala, B., Van den Bossche, P., Van den Abbeele, J., Delespaux, V., 2011. High prevalence of drug resistance in animal trypanosomes without a history of drug exposure. PLoS Negl. Trop. Dis. 5, e1454, http://dx.doi.org/10.1371/journal.pntd.0001454.

Clausen, P.H., Sidibe, I., Kabore, I., Bauer, B., 1992. Development of multiple drug resistance of *Trypanosoma congolense* in zebu cattle under high natural tsetse fly challenge in the pastoral zone of Samorogouan, Burkina Faso. Acta Trop. 51, 229–236.

Courtin, F., Rayaisse, J.B., Tamboura, I., Serdebeogo, O., Koudougou, Z., Solano, P., Sidibe, I., 2010. Updating the Northern tsetse limit in Burkina Faso (1949–2009): impact of global change. Int. J. Environ. Res. Public Health 7, 1708–1719.

de Almeida, P.J.L.P., Ndao, M., Vanmeirvenne, N., Geerts, S., 1997. Diagnostic evaluation of PCR in goats experimentally infected with *Trypanosoma vivax*. Acta Trop. 66, 45–50.

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. Vet. Parasitol. 117, 185–193.

Delespaux, V., Geysen, D., Van den Bossche, P., Geerts, S., 2008. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol. 24, 236–242.

Delespaux, V., Vitouley, H.S., Marcotty, T., Speybroeck, N., Berkvens, D., Roy, K., Geerts, S., Van den Bossche, P., 2010. Chemosensitization of *Trypanosoma congolense* strains resistant to isometamidium chloride by tetracycline and fluoroquinolone. PLoS Negl. Trop. Dis. 4, e828, http://dx.doi.org/10.1371/journal.pntd.0000828.

Gall, Y., Woitag, T., Bauer, B., Sidibe, I., McDermott, J., Mehlitz, D., Clausen, P.H., 2004. Trypanocidal failure suggested by PCR results in cattle field samples. Acta Trop. 92, 7–16.

Gardiner, P.R., 1989. Recent studies of the biology of *Trypanosoma vivax*. Adv. Parasitol. 28, 229–317.

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. Vet. Parasitol. 110, 171–180.

Grace, D., Randolph, T., Affognon, H., Dramane, D., Diall, O., Clausen, P.H., 2009. Characterisation and validation of farmers' knowledge and practice of cattle trypanosomosis management in the cotton zone of West Africa. Acta Trop. 111, 137–143.

Knoppe, T.N., Bauer, B., McDermott, J.J., Peregrine, A.S., Mehlitz, D., Clausen, P.H., 2006. Isometamidium sensitivity of *Trypanosoma congolense* stocks from cattle in West Africa tested in mice and the drug incubation infectivity test. Acta Trop. 97, 108–116.

Liebenehm, S., Affognon, H., Waibel, H., 2011. Collective livestock research for sustainable disease management in Mali and Burkina Faso. Int. J. Agr. Sustain. 9, 212–221.

Mamoudou, A., Delespaux, V., Chepnda, V., Hachimou, Z., Andrikaye, J.P., Zoli, A., Geerts, S., 2008. Assessment of the occurrence of trypanocidal drug resistance in trypanosomes of naturally infected cattle in the Adamaoua region of Cameroon using the standard mouse test and molecular tools. Acta Trop. 106, 115–118.

Masumu, J., Marcotty, T., Geysen, D., Geerts, S., Vercruysse, J., Dorny, P., Van den Bossche, P., 2006. Comparison of the virulence of *Trypanosoma congolense* strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. Int. J. Parasitol. 36, 497–501.

McDermott, J.J., Woitag, T., Sidibe, I., Bauer, B., Diarra, B., Ouedraogo, D., Kamuanga, M., Peregrine, A.S., Eisler, M.C., Zessin, K.H., Mehlitz, D., Clausen, P.H., 2003. Field studies of drug-resistant cattle trypanosomes in Kenedougou Province, Burkina Faso. Acta Trop. 86, 93–103.

Murray, M., Murray, P.K., Mcintyre, W.I.M., 1977. Improved parasitological technique for diagnosis of African Trypanosomiasis. Trans. R. Soc. Trop. Med. Hyg. 71, 325–326.

Van den Bossche, P., Doran, M., Connor, R.J., 2000. An analysis of trypanocidal drug use in the Eastern Province of Zambia. Acta Trop. 75, 247–258.