

## Tick control practices in Burkina Faso and acaricide resistance survey in *Rhipicephalus (Boophilus) geigy* (Acari: Ixodidae)

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Received: 27 July 2012 / Accepted: 3 September 2012 / Published online: 12 September 2012  
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**Abstract** Traditional systems account for 95 % of the livestock produced in Burkina Faso. Tick infestation hampers livestock productivity in this area. However, little information exists on tick-control practices used by livestock farmers. We interviewed 60 stockbreeders working in traditional farming systems to obtain the first data on tick-control practices. Sixteen farmers (27 %) did not use conventional practices: seven removed ticks by hand or plastered cattle with dung or engine oil; nine farmers treated cattle with crop pesticides. Forty-four farmers (73 %) used mainly synthetic pyrethroids (SP; either alphacypermethrin or deltamethrin in 20 and 18 farms, respectively) and occasionally amitraz (N = 6). Intervals between treatments varied significantly depending on the chemical used: most farmers using crop pesticides (100 %), amitraz (100 %) or alphacypermethrin (80 %) adjusted tick-control to tick-burden, whereas farmers using deltamethrin tended more to follow a tick-control schedule. Perception of tick-control effectiveness significantly varied among practices: tick-control failures were more frequently reported by farmers using alphacypermethrin (55 %) than by those using either other conventional acaricides (17 %) or crop pesticides (0 %). We investigated whether this could indicate actual development of SP-resistance in cattle ticks. First, using the larval packet test technique, we confirmed that the computation of LC<sub>50</sub> and LC<sub>90</sub> was repeatable and remained stable across generations of the *Rhipicephalus (Boophilus) geigy* Houndé laboratory strain. We then collected from the field fully-engorged female *R. geigy*

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to evaluate the SP-resistance relative to the Houndé reference strain. We did not detect any case of SP-resistance in the field-derived *R. geigy* ticks.

**Keywords** *Rhipicephalus (Boophilus) geigy* · Alphacypermethrin · Deltamethrin · Resistance · Larval packet test · Burkina Faso

## Introduction

In Burkina Faso, livestock production contributes 12 % to the gross domestic product and generates 19 % of the export earnings (Anonymous 2003, 2004). However, this economic sector is mainly characterized by its low productivity resulting from animal diseases, weak performance of the local breeds and from the nature of the production system (Anonymous 2003). Livestock production corresponding to semi-intensive farming systems where exotic breeds are used to improve animal production remains marginal: the corresponding farms, mainly located in urban and peri-urban areas, comprise only 5 % of the total livestock production (Anonymous 2003). Traditional, extensive and low-input systems contribute 95 % of the total livestock production. This includes the transhumant systems where partial or complete cattle herds move south to find forage in the dry season (i.e., from November to April) and return north in mid-May when the rainy season starts (Nianogo and Somda 1999). Traditional farming systems also include sedentary systems where sheep, goats and indigenous cattle (Zebu or N'Dama breed) are foraging together on communal pastures during rainy seasons and are kept under zero grazing in dry seasons.

With such a predominance of low-input farming systems within a country that has not reached food self-sufficiency, the evaluation of any measure contributing to the protection of animal health is of prime importance. This includes the evaluation of tick-control practices as ticks constitute major constraints to the livestock industry in tropical countries because of the direct losses they cause and the diseases they transmit (Sutherst and Kerr 1987; Jongejan and Uilenberg 1994). In West Africa, *Amblyomma variegatum* (Fabricius) is considered the most harmful tick species (Mattioli et al. 1997; Knopf et al. 2002; Farougou et al. 2007). This tick impairs animal growth (Pegram et al. 1989; Pegram and Oosterwijk 1990; Stachurski et al. 1993) and exacerbates dermatophilosis (Plowright 1956). Moreover it transmits to ruminants the causative agent of the deadly disease cowdriosis or heartwater (*Ehrlichia ruminantium*). The *A. variegatum* adults, which are active during the rainy seasons, are thus the priority targets for tick-control practices (Stachurski 2000). Smallholders practising low-input traditional farming used to remove ticks by hand. They are increasingly using pesticides but without close supervision by veterinary services, so that one cannot rule out the possibility that chemical misuses occur and facilitate the development of acaricide resistance in cattle ticks.

We addressed this issue by performing the first survey of tick-control practices in Burkina Faso. We interviewed 60 farmers throughout the country about their tick-control practices; of these 58 practiced the predominant extensive, traditional low-input system. We also investigated the resulting impact of tick-control practices on the putative development of acaricide resistance in cattle ticks. To do so, we focused on a species ubiquitous in Burkina Faso, with a very short generation time, which thus can serve as a sentinel species: *Rhipicephalus (Boophilus) geigy*. Although of lesser importance than *A. variegatum*, *R. (Boophilus) geigy* is also of practical importance due to the role it plays as *Babesia bovis* vector's (Akinboade and Dipeolu 1981). Baselines for acaricide-induced mortality were provided by one *R. geigy* strain that has been maintained at CIRDES

laboratory facilities since 2005. These baselines were compared to the acaricide-induced mortalities observed in the offspring of fully engorged female *R. geigy* ticks collected on some of the farms where farmers were interviewed about their tick control practices.

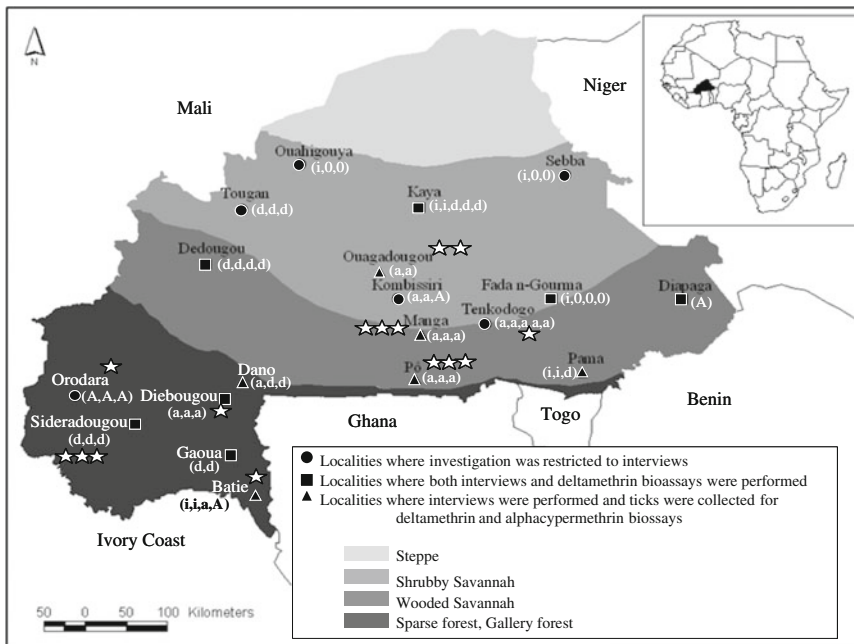
## Materials and methods

### Study area and production systems

The study was carried out in Burkina Faso, West Africa (10–15°N; 2–5°E), between the northern Sahelian and southern sudano-guinean climatic areas (Fig. 1). Two farms located near Ouagadougou practised a semi-intensive production system; the 58 others practised the traditional and low-input farming system.

### Investigation of tick control practices

The questionnaire submitted to farmers included five main questions relative to tick-control practices: 1—Do you practice tick-control? 2—If so, which products do you use? 3—How do you apply tick-control? 4—What is the average frequency of acaricide treatment? 5—Do you perceive tick-control as efficient?



**Fig. 1** Sites for tick sampling and survey of tick control practices in Burkina Faso. The chemical usage reported by each interviewed farmer is indicated in parentheses (0 no chemical usage, *i* insecticides designed for crop protection, *a* alphacypermethrin, *d* deltamethrin, *A* amitraz). Stars represent farmers reporting tick-control failures

### Laboratory reference strain

Fifty fully engorged *R. geigy* females were collected from a cattle herd from the Houndé district in February 2005. Following transfer to the CIRDES laboratory, ticks were placed under optimal conditions of temperature ( $27 \pm 2$  °C) and relative humidity ( $85 \pm 5$  %) for egg-laying. The first batches of eggs were maintained in an incubator at these same controlled conditions until they hatched. The resulting larvae were assessed for alphacypermethrin-induced mortality when they were 9–20 days old and for deltamethrin-induced mortality when they were 8–23 days old. The remaining  $F_0$  ticks were allowed to infest a Zebu  $\times$  N'Dama crossbred steer. From then onwards, each successive *R. geigy* generation was completed in less than 93 days: larvae were placed on cattle within 45 days after hatching and the non-parasitic period (i.e., the time required for females to lay eggs and for eggs to hatch) was limited to 27 days. The steer used for infestation was replaced every three tick generations. We assessed synthetic pyrethroid (SP)-induced mortality in Houndé strain ticks as follows: the alphacypermethrin-induced mortality at  $F_0$ ,  $F_1$ ,  $F_5$  and  $F_{12}$  generations, and the deltamethrin-induced mortality at  $F_0$ ,  $F_1$ ,  $F_2$  and  $F_{12}$ .

### Tick sampling for bioassays

*Rhipicephalus (Boophilus)* spp. ticks were found and collected at 13 of the localities visited (Fig. 1). Fully engorged females were sampled from the most heavily infested cows within each herd. We formed one sample per locality, by pooling together ticks collected on different cows within herds situated in close proximity to each other to make a single sample. This should not introduce strong bias since, in the traditional low input systems, herds from the same locality are foraging together on the same communal pastures. Ticks were placed in plastic tubes with a lid covered with tulle to be brought to CIRDES within the 2–5 days following sampling. Ticks were placed under optimal conditions as described above. Females were identified to species level by counting the denticles present on the hypostome (Walker et al. 2003). Whenever both male and female ticks were collected together, the examination of male morphology was performed to confirm the species identification of females.

### Bioassays

Bioassays were performed with the following synthetic pyrethroids as commercial formulations: deltamethrin (Butox<sup>®</sup>, SOFACO, Group Roussel Uclaf, Paris) and alphacypermethrin (Dominex<sup>®</sup>, FMC Corp, USA). The concentrations of the commercial products were 50 and 100 g/l, respectively. When the number of larvae obtained was insufficient to test both products, priority was given to deltamethrin.

The bioassay method used was the larval packet test (LPT) developed by Stone and Haydock (1962) and subsequently adopted by FAO as a standard (FAO 2004). Chemicals were diluted in a mix of olive oil and trichloroethylene in a 2:1 volume/volume ratio. Packets were formed from  $7.5 \times 8.5$  cm pieces of Whatman filter paper. Each piece of paper was first impregnated with 0.67 ml of acaricide solution (or solvent alone for the controls) and placed for 2 h in a fume hood for solvent evaporation. The papers were then folded to form packets within which we placed a minimum of 100 tick larvae. The larvae were 14–21 days old when used in the LPT. Each control and each acaricide dose were tested at least in duplicate. Each LPT involved 1,988 larvae on average (range: 1,556–3,892). The packets were then incubated at controlled temperature ( $27 \pm 2$  °C) and

relative humidity ( $85 \pm 5 \%$ ). Live and dead ticks were counted after 24 h of acaricide exposure.

Preliminary tests performed on the reference Houndé strain for both commercial SP acaricides identified the highest SP concentration inducing no mortality as 0.00312 g/l and the lowest concentration inducing 100 % mortality as 0.4 g/l. Accordingly, the LPT were defined by series of 8 half-dilution doses distributed in between these extremes. The active ingredients in diluted acaricide can deteriorate (Moreira et al. 2002) we thus prepared fresh acaricide dilutions at least monthly.

### Statistical analysis

We analyzed mortality responses using Polo-PC (LeOraSoftware 1987) according to the stepwise procedure described by Robertson and Preisler (1992). Abbott correction was unnecessary since mortality in controls always remained below 0.05. Mortalities were probit-transformed, and acaricide doses were transformed into their natural logarithm. We adjusted transformed data with a linear model in order to estimate  $LC_{50}$  (i.e. concentration resulting in 50 % SP-induced mortality). Deviation from linearity of log-probit transformed data was assessed through a Chi-square test (Robertson and Preisler 1992). We computed resistance ratios (RRs) and 95 % confidence intervals (95 % CI) relative to the reference Houndé strain by taking into account the variance and covariance of the slopes and intercepts of both regressions (Robertson and Preisler 1992). Difference between  $LC_{50}$  estimates was designated as significant if confidence intervals did not overlap.

## Results

### Tick control practices and perceived efficacy of tick-control

All the interviewed farmers affirmed that they only controlled ticks in the rainy season; i.e., from mid-May to October. Seven out of the 60 interviewed farmers (11 %) declared that they had no tick control program apart from manual removal and/or application of oil or dung on cattle (Fig. 1). Four of these were located in the dry northern part of the country, with two in Ouahigouya and two in Sebba where the mean rainfall is around 400 mm/year (Fig. 1). Three others were located in Fada N’Gourma (Fig. 1). Nine farmers (15 %) from the northern and western provinces controlled ticks by plastering cattle with insecticides that they diverted from their original use for crop protection against agricultural pests (Fig. 1). Finally, 44 stockbreeders (73 %) sprayed conventional acaricides on cattle: 20 sprayed alphacypermethrin, 18 sprayed deltamethrin and 6 sprayed amitraz. Interestingly, these differing acaricide usages did not appear to be randomly distributed throughout the country: the use of deltamethrin was seen mostly in the western districts while that of alphacypermethrin was more frequent in central districts (Fig. 1).

For the 53 farmers who based tick-control on pesticide chemicals, we regrouped the information gathered on treatment intervals and perception of tick-control efficacy in Table 1. The farmers’ decision on treatment intervals significantly varied with the nature of the chemical used in control tick (Fischer exact test on  $R \times C$  contingency tables;  $P = 0.0013$ ). The rule was to adjust treatment based on a subjective visual evaluation of tick burden among farmers using unconventional acaricides (100 %), among those using amitraz (100 %), as well as among farmers using alphacypermethrin (80 %). This was

however, not the case among farmers using deltamethrin: most of these farmers followed a priori determined schedules for chemical applications (61 %, Table 1).

The perceived tick-control efficacy also significantly varied with the nature of pesticides used for tick-control (Fischer exact test on  $R \times C$  contingency tables;  $P < 0.001$ ). Farmers diverting insecticides from crop protection to tick-control purposes were the only ones who did not report any tick-control failure. Heterogeneity in perceived tick-control efficacy remained significant when only considering the farmers using conventional acaricides (Fischer exact test on  $R \times C$  contingency tables;  $P = 0.04$ ): the frequency in the report of tick-control failures was 0.17 among farmers using amitraz, 0.17 among farmers using deltamethrin but 0.55 among those using alphacypermethrin. Interestingly however, for a given chemical use, whether treatments intervals resulted from tick-burden evaluation or from a priori fixed schedule did not impact the frequency in the reports of tick-control failures (Fischer exact test on  $R \times C$  contingency tables;  $P > 0.05$  in all cases).

#### Establishment of baseline acaricide susceptibility in the Houndé strain

Table 2 summarizes the results obtained for the Houndé strain. Fit of mortality data by the log-probit linear model was rarely perfect ( $\chi^2$ -test,  $P < 0.05$  in 8 out of 11 assays; Table 2). None of the successive generations deviated from the SP-mortality responses observed at the  $F_0$  generation (deltamethrin:  $0.93 \leq RR_{50} \leq 1.39$  and  $0.89 \leq RR_{90} \leq 1.04$  in all cases; alphacypermethrin:  $0.79 \leq RR_{50} \leq 1.14$  and  $0.45 \leq RR_{90} \leq 0.96$ , in all cases).

#### Susceptibility levels to deltamethrin and alphacypermethrin in field-derived larvae

Table 3 summarizes the results obtained for field-derived *R. geigy* larvae. The log-probit model did not perfectly fit the observed mortality data ( $\chi^2$ -test,  $P < 0.05$  in all cases except the alphacypermethrin-induced mortality observed in samples originating from

**Table 1** Chemical tick control in Burkina Faso: practices and perceived efficacy

Practices and number of farms concerned			Control failures perceived by stockbreeders	
Chemical	Application	Intervals between treatments	N	Farms localization
Insecticides (crops)	Plastering (N = 9)	Adjusted to tick burden (N = 9)	0	
Deltamethrin	Spray (N = 18)	Adjusted to tick burden	3	Sidéradouougou
		14 days (N = 5)	0	
		7–10 days (N = 6)	0	
Alphacypermethrin	Spray (N = 20)	Adjusted to tick burden	9	Diébougou, Manga, Ouagadougou, Pô, Tenkodogo
		14 days (N = 2)	1	Batié
		7–10 days (N = 2)	1	Ouagadougou
Amitraz	Spray (N = 6)	Adjusted to tick burden (N = 6)	1	Orodara

Tick control practices are described by the chemical used, the application methods and method used to determine treatment intervals (the total number of identical answers figure in parentheses). The number of farmers who reported tick-control failures and the location of their farms are reported

**Table 2** Stability in SP-induced mortality in the *Rhipicephalus geigy* Houndé strain

Chemical	Date (dd/mm/yy)	Generation	Slope $\pm$ SE	LC <sub>50</sub> (95 % CI)
Deltamethrin	26/04/2005	F <sub>0</sub>	3.6 $\pm$ 0.1*	0.039 (0.035–0.044)
	12/07/2005	F <sub>1</sub>	5.3 $\pm$ 0.3	0.055 (0.052–0.057)
	13/07/2005	F <sub>1</sub>	3.8 $\pm$ 0.1*	0.043 (0.041–0.046)
	15/10/2005	F <sub>2</sub>	3.6 $\pm$ 0.2*	0.036 (0.032–0.041)
	13/10/2008	F <sub>12</sub>	3.9 $\pm$ 0.2	0.041 (0.038–0.044)
Alphacypermethrin	13/04/2005	F <sub>0</sub>	2.9 $\pm$ 0.1*	0.038 (0.032–0.045)
	19/04/2005	F <sub>0</sub>	3.3 $\pm$ 0.1*	0.045 (0.041–0.049)
	15/07/2005	F <sub>1</sub>	3.9 $\pm$ 0.1	0.036 (0.034–0.039)
	19/07/2005	F <sub>1</sub>	4.0 $\pm$ 0.1*	0.029 (0.027–0.031)
	22/06/2006	F <sub>5</sub>	3.4 $\pm$ 0.1*	0.047 (0.042–0.055)
	14/10/2008	F <sub>12</sub>	3.9 $\pm$ 0.2*	0.033 (0.030–0.036)

Slopes are estimated around LC<sub>50</sub>; slope estimates are followed by a star if the Chi-square tests of the goodness of fit reject linearity ( $P < 0.05$ ). LC<sub>50</sub> are expressed in g/l

Dano and Manga). The deltamethrin-susceptibility was generally higher in field-derived samples than in the Houndé laboratory strain ( $RR \leq 1$ ; Table 2) except for the Sidéradougou sample ( $1 < RR_{50} < 1.6$  and  $1 < RR_{99} < 1.5$ ). In alphacypermethrin-bioassays, all field-derived larvae were as susceptible or even more susceptible ( $RR_{50} < 1$  and  $RR_{99} < 1$ ) than the Houndé laboratory strain.

## Discussion

The present study is the first to document tick-control practices in Burkina Faso, and confirmed that tick-control practices greatly vary throughout the country. Simple hypotheses may explain part of the observed heterogeneity. For instance, the manual removal of ticks and the misuse of agricultural insecticides for tick-control purposes were more frequent in the driest regions of the country. This is unlikely to be coincidental as *A. variegatum*, which is the tick species associated with the heaviest economic losses in West Africa (Mattoli et al. 1997; Knopf et al. 2002; Farougou et al. 2007), is less abundant in these driest regions (Stachurski 2000). Geographical heterogeneity in the supply of products to local markets may also contribute to the heterogeneity in the chemical used in tick-control. This may be one of the factors explaining why amitraz used in tick control appeared minimal in Burkina Faso as a whole but was predominant in Orodora (Fig. 1). In fact Orodora is located near to South Mali where amitraz is the only chemical used in tick-control (Adakal, unpublished data).

The variation in the perceived efficacy of tick control practices is puzzling. Given the short generation time of *R. geigy* (at least 3–4 generations per year) it would be very unlikely that the farmers' tick control practices promoted acaricide resistance in other cattle tick species without promoting any in *R. geigy*. As we failed to detect any resistance in field-collected *R. geigy* samples to the SP-chemicals used by farmers in Burkina Faso, the reports of tick control failures by farmers who controlled ticks with deltamethrin or alphacypermethrin are unlikely to indicate acaricide resistance. Moreover, farmers who diverted agricultural insecticides for tick-control purposes could be suspected of answering bias: these farmers were perfectly aware that this was considered as chemical misuse and might thus have been more prone than other interviewees to hide any pesticide misuse or tick-control difficulty.

**Table 3** SP-bioassays in field-derived *Rhipicephalus geigy* ticks

Chemical	Sample	Slope $\pm$ SE	LC <sub>50</sub> (95 % CI)	RR <sub>99</sub> (95 %CI)
Deltamethrin	Houndé-strain	3.4 $\pm$ 0.1*	0.036 (0.028–0.047)	–
	Batié	4.0 $\pm$ 0.1*	0.025 (0.023–0.027)	0.55 (0.46–0.67)
	Dano	3.9 $\pm$ 0.1*	0.031 (0.028–0.034)	0.70 (0.58–0.87)
	Débougou	3.5 $\pm$ 0.1*	0.034 (0.031–0.038)	0.90 (0.73–1.12)
	Diapaga	4.6 $\pm$ 0.2*	0.026 (0.024–0.028)	0.48 (0.40–0.60)
	Diébougou	4.4 $\pm$ 0.2*	0.027 (0.024–0.030)	0.53 (0.44–0.65)
	Fada N'Gourma	3.3 $\pm$ 0.1*	0.035 (0.032–0.040)	1.06 (0.86–1.32)
	Gaoua	5.5 $\pm$ 0.1*	0.017 (0.016–0.020)	0.27 (0.23–0.33)
	Kaya	4.1 $\pm$ 0.2*	0.041 (0.037–0.045)	0.89 (0.73–1.10)
	Kombissiri	4.1 $\pm$ 0.2*	0.027 (0.024–0.032)	0.59 (0.48–0.74)
	Manga	5.5 $\pm$ 0.3*	0.019 (0.018–0.021)	0.29 (0.24–0.36)
	Ouagadougou	4.8 $\pm$ 0.2*	0.031 (0.028–0.034)	0.55 (0.45–0.68)
	Pama	3.8 $\pm$ 0.1*	0.027 (0.026–0.029)	0.65 (0.55–0.79)
	Pô	17.3 $\pm$ 1.1*	0.012 (0.011–0.012)	0.09 (0.08–0.11)
	Sidéradougou	3.8 $\pm$ 0.2*	0.052 (0.045–0.061)	1.25 (1.02–1.56)
Alphacypermethrin	Houndé-strain	3.9 $\pm$ 0.2*	0.031 (0.029–0.033)	–
	Batié	3.4 $\pm$ 0.3*	0.023 (0.020–0.025)	0.89 (0.72–1.11)
	Dano	5.0 $\pm$ 0.2*	0.018 (0.017–0.019)	0.44 (0.37–0.54)
	Manga	5.1 $\pm$ 0.2	0.021 (0.020–0.023)	0.50 (0.42–0.61)
	Ouagadougou	3.0 $\pm$ 0.1*	0.022 (0.019–0.024)	1.05 (0.85–1.30)
	Pama	3.3 $\pm$ 0.1*	0.022 (0.020–0.025)	0.91 (0.74–1.14)
	Pô	6.6 $\pm$ 0.4*	0.013 (0.012–0.014)	0.24 (0.20–0.29)

Slopes are estimated around LC<sub>50</sub>; slope estimates are followed by a star if the Chi-square tests of the goodness of fit reject linearity ( $P < 0.05$ ). LC<sub>50</sub> are expressed in g/l. RR<sub>99</sub> are expressed relative to the *R. geigy* Houndé strain

The Houndé strain results from the first attempt in West Africa to establish a local *Rhipicephalus (Boophilus) sp.* reference strain for monitoring the possible development of acaricide resistance in cattle ticks. The observed stability in the Houndé strain SP-mortality responses across generations indicates a homogeneous genetic composition for this strain. This is also indicative of a good standard of quality and repeatability for the LPT performed at CIRDES. This will be of great help in the near future for helping farmers to protect their livestock against currently changing tick-associated risks. Introduced a few years ago into Benin and Ivory Coast (Madder et al. 2007, 2012), *Rhipicephalus (Boophilus) microplus* was found to have started to invade these countries while displacing the indigenous *Rhipicephalus (Boophilus)* species there (Madder et al. 2011). Moreover, the occurrence of invasive *R. microplus* populations in Ivory Coast resulted in tick-control failures, inappropriate pesticide use and increased animal mortality (Madder et al. 2011). Finally, based on farmers' claims about acaricides ineffectiveness in the field, *R. microplus* is suspected to occur in Mali and Burkina Faso along their boundaries with Ivory Coast (Adakal, unpublished results). Therefore, further acaricide resistance surveys will be required immediately in Burkina Faso to help the local farmers to optimize animal health protection against this and other tick-associated risks.

**Acknowledgments** Funding for this work was provided by the French Government through the Fonds de Solidarité Prioritaire (FSP) under contract no 2000-113. We thank the Burkina Faso Veterinary Services for



tick collection assistance, Sébastien Zoungrana and Maurice Konkobo for laboratory assistance. Special thanks to Dr. Lesley Bell-Sakyi, Dr. Peter Willadsen and Dr. Maxime Madder for their valuable and useful comments on the manuscript.

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