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Resistance of the cattle tick *Rhipicephalus microplus* to alphacypermethrin, deltamethrin and amitraz in Côte d'Ivoire

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ABSTRACT

Cattle tick *Rhipicephalus microplus* has become a common problem for cattle herders in Côte d'Ivoire after its discovery there in the early 2000s, despite the availability of a large range of acaricide products. The objective of this study was to assess the resistance of the cattle tick *R. microplus* to alphacypermethrin, deltamethrin and amitraz, and to provide information to advice farmers of acaricide products choice in Côte d'Ivoire. A cross-sectional survey was conducted in September 2016 on nine farms where resistance to acaricides was suspected. Engorged *R. microplus* females were sampled and transported to the International Centre for Research and Development on Subhumid Livestock Production (CIRDES) in Burkina Faso. The FAO larval packet test method (adapted by CIRDES) was applied to assess the resistance-susceptibility level of the *R. microplus* tick populations to three common acaricidal molecules (alphacypermethrin, deltamethrin and amitraz) in Côte d'Ivoire. The results showed resistance ratios (RR) at 50% (RR50 95% CI) ranging from 0.26 (0.20-0.33) to 1910.3 (0.0-9063.3). High resistance to amitraz and deltamethrin was found on all farms, whereas resistance of tick populations to alphacypermethrin varied by farm from very susceptible to highly resistant. These results

indicate that alphacypermethrin should remain a recommended acaricide on some farms in the country and underline the importance of raising awareness on the appropriate use of acaricides and monitoring acaricide resistance in tick populations in Côte d'Ivoire. Such actions will support farmers in the control and prevention of *R. microplus* infestation in cattle herds in Côte d'Ivoire.

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Keywords: *Rhipicephalus microplus*, resistance, acaricides, Larval Packet Test, Côte d'Ivoire.

INTRODUCTION

Livestock farming in sub-Saharan Africa faces several limitations that affect its performance and hinder its development. Among these constraints, ticks and related transmitted diseases are particularly important. For decades, tick-control by West African livestock owners has focused on limiting the damage caused by the tick species *Amblyomma variegatum* (Farougou et al., 2007). This tick is the indigenous tick species to Africa and is endemic across the Sub-Saharan Africa. However, the emergence of the exotic tick *Rhipicephalus microplus* (*R. microplus*) has emerged in Ivorian cattle herds in the early 2000s, has overtaken indigenous tick species. This tick species, associated with the greatest economic losses worldwide (Ghosh et al., 2007) is specific to cattle and transmits *Babesia bovis* and *Babesia bigemina* that cause bovine babesiosis, which results in significant morbidity and mortality. *Rhipicephalus microplus* is also known to be resistant to several of the commonly used acaricide treatments (Guerrero et al., 2012; Kumar et al., 2020). It has developed resistance to 43 different acaricide molecules, making it one of the 20 arthropod species with the broadest resistance spectra (Whalon et al., 2008). This large spectrum of resistance is also a consequence of the misusing of acaricides by farmers. *Rhipicephalus microplus* represents a major threat to livestock producers, particularly farmers in low resource settings who depend on livestock farming. Following the introduction of *R. microplus* in the south of Côte d'Ivoire in 2002-2004, it has quickly spread throughout the country (Toure et al., 2012; Toure et al., 2014; Boka et al., 2017). The seasonal variation of this tick is also

known in the north, centre and south of the country as it has been reported in Mali (Diarra et al., 2017), Benin and Burkina Faso (Biguezoton et al., 2016). Amitraz, deltamethrin or alphacypermethrin are the acaricide products generally used to control ticks, but faced recurrent therapeutic failures and complaints from farmers (Adehan et al., 2016). Therefore, this study aimed at assessing the resistance of the cattle tick *R. microplus* to alphacypermethrin, deltamethrin and amitraz, and to provide information to advice farmers of acaricide products choice in Côte d'Ivoire.

MATERIALS AND METHODS

Study area

The study was carried out in the largest cattle breeding areas of Côte d'Ivoire, in locations where *R. microplus* is known to infest cattle (Figure 1). Nine farms were visited, in the South, Centre and North of the country. The following locations were considered: Azaguié (three farms) and Bingerville (one farm) in the South, Dabakala (one farm) and Toumodi (one farm) in the Centre and Korhogo (one farm) and Ferkessédougou (two farms) in the North (Figure 1).

Cattle tick sampling

In a cross-sectional survey conducted in September 2016, engorged *R. microplus* female ticks were collected from cattle on nine farms (Table 1) where tick resistance to acaricides was suspected. Sampling was implemented at least two weeks after the last acaricide treatment. Tick collection was carried out on cattle over one year of age and between 9 to 11 AM, to increase the likelihood of collecting well-engorged females. The herdsmen using a rope restrained the cattle in

the lateral decubitus position and half-body counts were performed for 10 minutes, in the tick predilection areas, namely: the ear, the dewlap, the buttocks, the flank, the udder, the perineum, the tail and the inner side of the thigh. The collection was carefully done to avoid to damage the tick by heavy pressure or the hypostome when detaching it. The engorged females of *R. microplus* collected were then stored in specially prepared bottles, which were clearly identified and placed on a tray containing a clean cloth soaked in water to maintain the wet environment and thus allow the ticks to survive. The lids of the vials and the trays were perforated and protected by a net and a fine-mesh screen in order to provide air for the ticks and to avoid they escape.

Date, name of the breeder, host breed, age and ID number, if possible, were recorded for each sample. The females, which were kept alive, were taken to the acarology laboratory of the International Centre for Research and Development on Subhumid Livestock Production (CIRDES) in Bobo-Dioulasso, Burkina Faso. The unfed *R. microplus* female and other tick species (whatever their stage of development) were packaged in tubes containing 70% ethanol and sent to the laboratory for species identification.

Laboratory analysis

Identification of ticks

The identification of *R. microplus* females was confirmed based on morphological approach. Ticks were examined using a magnifying glass (SMZ745T, NIKON, France), and identified based on the identification key developed for ticks of the subgenus *Rhipicephalus* (*Boophilus*) (Walker, 2003).

Tick rearing

The female ticks were kept in an incubator at 27°C, with a relative humidity of 80 to 90% until eggs were laid. The eggs were then placed in the incubator at a temperature of 27 ± 1°C with a relative humidity of 90 to 95%, conditions allowing good hatching. The larvae were kept for 14 – 21 days before being used

for testing. As the number of engorged females collected was not the same in the farms, the number of eggs and thus the number of larvae recorded after laying were not the same for the samples (Table 1).

Resistance test

The three most commonly used acaricide molecules in Côte d'Ivoire, namely amitraz, alphacypermethrin and deltamethrin were used to assess resistance. The analyses of the resistance of ticks to acaricides were carried out using the "larval packet test" (LPT) method according to the FAO protocol adapted by the CIRDES (Adakal et al., 2013). The larvae obtained were treated with a series of dilutions (Table 2) of each acaricide to be tested. Different concentrations with a mix ratio of 1 volume of olive oil for 2 volumes of trichloroethylene (Miller et al., 2002) as solvent were applied. Whatman filter paper was then cut into packets of 7.5 X 8.5 cm size and 0.67 ml of each acaricide concentration was applied on each piece of paper filter. The assays were performed twice (in duplicate). For each test, two filter papers were impregnated only with the solvent (combination of olive oil and trichloroethylene) and used as controls. Impregnated packets were placed for 2 hours in a fume hood for evaporation. They were then filled with about 100 larvae of samples and incubated at 27 ± 2°C with 85 ± 5% relative humidity. After 24 h of acaricide exposure, larvae able to move were considered alive, and non-moving one were considered dead. They were counted and mortality rate (dead /total) was computed for each group/concentration. Thereafter, the induced larval mortality rate was compared with the mortality rate of a susceptible CIRDES reference strain, the *Rhipicephalus geigy* strain (Adakal et al., 2013). This strain is reared in the acarology laboratory of CIRDES. Over the years, its generations are renewed by artificial infestations on cattle.

Statistical analysis

The package Dose-Response Curves (drc) (Ritz et al., 2015) was used to compute a

non-linear regression analysis of dose-mortality data in R (R Core Team, 2013). Prior to the lethal dose (LD) estimates, models that fit better to analysed data were selected based on the lowest residual variance through the function `mselect` (Ritz et al., 2015). Thereafter, four parameters (b: slope, c: lower value, d: upper value and e: ED50) were computed with generalized log-logistic models with functions `LL.4` (amitraz and deltamethrin) and `LL.5` (alphacypermethrin). Lethal concentrations LD_{50} and LD_{90} (doses that can

cause the death of 50 and 90% of larvae respectively) as well as the Resistance Ratios (RR) were determined with a 95% confidence interval. The RRs corresponds to the ratio of the lethal concentration of a given sample to that of the reference susceptible strain. The different ratios obtained made it possible to determine the resistance status. A sample was considered susceptible if $RR < 4$, moderately resistant if $4 < RR < 10$ and highly resistant if $RR > 10$ (Lovis et al., 2013).

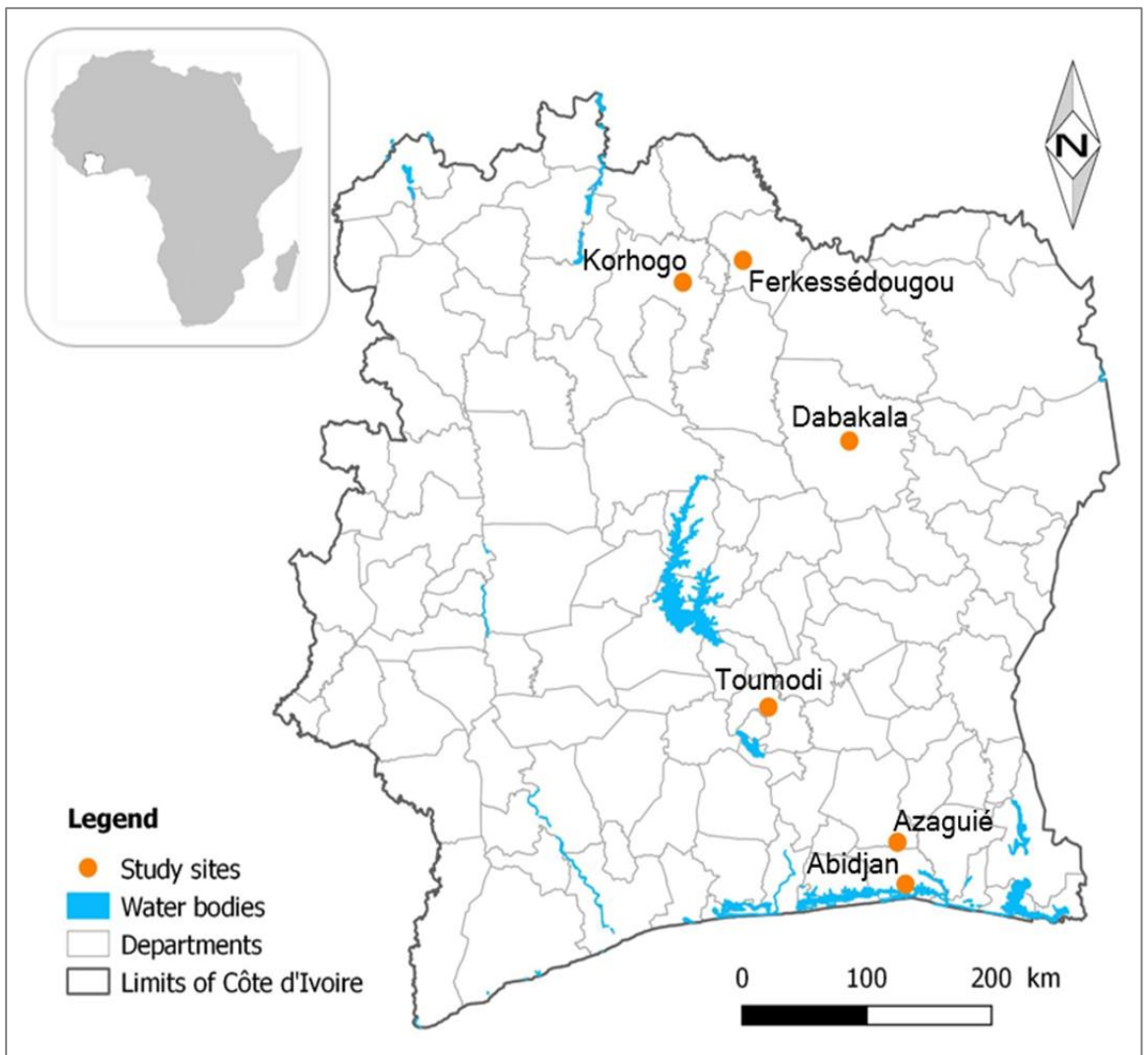


Figure 1: Study area.

Table 1: Samples collected and acaricides tested per farm.

Localities	Farms	Collected ticks	Acaricides tested
Azaguié (South)	A	12 well engorged females	Amitraz
		16 medium engorged	Deltamethrin Alpha cypermethrin
	B	23 well engorged females	Amitraz Deltamethrin Alpha cypermethrin
Bingerville (South)	D	16 well engorged females	Amitraz
		8 medium engorged	Deltamethrin
Toumodi (Centre)	E	20 well engorged females	Amitraz
		5 medium engorged	Deltamethrin
Dabakala (Centre)	F	10 well engorged females	Amitraz
		39 medium engorged	Deltamethrin
Korhogo (North)	G	12 well engorged females	Amitraz
		34 medium engorged	Deltamethrin Alpha cypermethrin
Ferkessédougou (North)	I	19 well engorged females	Amitraz
		7 medium engorged	Deltamethrin
	J	11 well engorged females 51 medium engorged	Amitraz

Table 2: Analysis samples and series of dilutions of the acaricidal products used.

Acaricides	Active principle	Samples/Location	Dilution series (%)
Antitic (amitraz 12.5%)	Amitraz	Azaguie_A/South Azaguie_C/South Azaguie_B/South Binger/South	0.0000312; 0.0000625; 0.000125; 0.00025; 0.0005; 0.001; 0.002; 0.004

		Dabak/Centre	
		Toumodi/Centre	
		Ferke_A/North	
		Ferke_B/North	
		Korhogo/North	
Alphacypermethrin 10% EC	Alphacypermethrin	Azaguie_A/South	
		Azaguie_B/South	
		Dabak/Centre	
Deltamethrin 5% EC	Deltamethrin	Azaguie_A/South	
		Azaguie_B/South	
		Binger/South	0.00312; 0.00625; 0.0125;
		Dabak/Centre	0.025; 0.05; 0.1; 0.2; 0.4
		Toumodi/Centre	
		Ferke_B/North	
Antitic (amitraz 12,5%)	Amitraz		0.00000312; 0.00000625;
			0.0000125; 0.000025;
			0.00005; 0.0001; 0.0002;
		Houde/Burkina Faso	0.0004
Alphacypermethrin 10% EC	Alphacypermethrin		0.00312; 0.00625; 0.0125;
Deltamethrin 5% EC	Deltamethrin		0.025; 0.05; 0.1; 0.2; 0.4

RESULTS

The results of the analyses are summarized in Table 3 and Figures 2, 3 and 4. The 50% resistance ratios (RR50 95% CI) range from 18.07 (14.16 - 21.98) to 108.69 (93.18 - 124.19) for amitraz, 0.26 (0.20 - 0.33) to 12.22 (0.0 - 32.66) for alphacypermethrin and 89.96 (42.966 - 136.95) to 1910.3 (0.0 - 9063.3) for deltamethrin. The samples tested appear to be very resistant to amitraz and deltamethrin (Table 3; Figures 2 and 3). On the other hand, the resistance varied for alphacypermethrin depending on the farm of origin (Table 3; Figure 4). For the three

samples tested with alphacypermethrin, the results indicate that the sample "Azaguie_B" is highly resistant, the sample "Azaguie_A" is moderately resistant and the sample "Dabak" is highly resistant to alphacypermethrin (Table 3). In addition, acaricide products influence also resistance status. For instance, "Azaguie_A" sample is highly resistant to amitraz and deltamethrin while its resistance to alphacypermethrin is moderate. Similarly, "Dabak" sample is highly resistant to amitraz and deltamethrin but more susceptible to alphacypermethrin than the reference strain (Table 3).

Table 3: Lethal concentrations and resistance status of samples.

Sample/origin	LC ₅₀ (CI) (% EC)	RR ₅₀ (CI)	LC ₉₀ (CI) (% EC)	RR ₉₀ (CI)	Slope	Resistance status
Amitraz 12,5% EC						
Houde (Burkina Faso)	7.20e-06 (6.41e-06 – 1.00e-05)	-	1.87e-05 (1.36e-05 - 1.00e-04)	-	-2.40±0.61	-
Azaguie_A	7.83e-04 (7.10e-04 – 9.00e-04)	108.69 (93.18 - 124.19)	1.49e-03 (9.72e-04 - 2.00e-03)	79.45 (44.64 - 114.26)	-4.14±2.25	Resistant
Azaguie_C	4.07e-04 (3.71e-04 - 4.00e-04)	56.50 (48.61 - 64.39)	7.61e-04 (5.42e-04 - 1.00e-03)	40.67 (24.65 - 56.70)	-4.29±2.04	Resistant
Azaguie_B	5.99e-04 (5.24e-04 - 7.00e-04)	83.14 (69.37 - 96.91)	1.42e-03 (9.23e-04 - 2.00e-03)	75.80 (42.40 - 109.20)	-4.40±2.74	Resistant
Binger	5.09e-04 (4.50e-04 - 6.00e-04)	70.73 (59.41 - 82.05)	1.20e-03 (8.74e-04 - 1.50e-03)	64.13 (39.59 - 88.66)	-3.42±1.22	Resistant
Dabak	1.30e-04 (1.06e-04 - 2.00e-04)	18.07 (14.16 - 21.98)	4.09e-04 (3.36e-04 - 5.00e-04)	21.88 (14.7802 - 28.98)	-14.07±11.72	Resistant
Toumodi	2.40e-04 (2.06e-04 - 3.00e-04)	33.27 (27.42 - 39.12)	5.04e-04 (4.55e-04 - 6.00e-04)	26.94 (19.20 - 34.68)	-16.81±16.06	Resistant
Ferke_A	2.50e-04 (2.23e-04 - 3.00e-04)	34.66 (29.42 - 39.91)	4.92e-04 (4.01e-04 - 6.00e-04)	26.30 (17.69 - 34.91)	-4.79±1.77	Resistant
Ferke_B	1.79e-04 (1.51e-04 - 2.00e-04)	24.88 (20.17 - 29.60)	4.55e-04 (3.95e-04 - 5.00e-04)	24.34 (17.01 - 31.67)	-12.70±12.78	Resistant
Korhogo	2.88e-04 (2.61e-04 - 3.00e-04)	40.01 (34.25 - 45.77)	4.92e-04 (4.16e-04 - 6.00e-04)	26.30 (18.11 - 34.49)	-5.40±2.09	Resistant

Alphacypermethrin 10% EC						
Houde (Burkina Faso)	0.03 (0.03 - 0.04)	-	0.07 (0.05 - 0.08)	-	-3.07±0.38	-
Azaguie_A	0.20 (0.18 - 0.22)	5.93 (5.16 - 6.70)	0.33 (0.21 - 0.45)	4.83 (2.87 - 6.79)	-4.30±1.29	Moderately Resistant
Azaguie_B	0.4 (0.0 - 1.09)	12.22 (0.0 - 32.66)	1.74 (0.0 - 6.34)	25.60 (0.0 - 92.72)	-1.51±0.51	Resistant
Dabak	0.0 (0.01 - 0.01)	0.26 (0.20 - 0.33)	0.06 (0.03 - 0.10)	0.94 (0.37 - 1.51)	-1.10±0.13	Sensitive
Deltamethrin 5% EC						
Houde (Burkina Faso)	2.15e-03 (1.07e-03 - 3.20 e-03)	-	3.72e-03 (2.77e-03 - 0.005)	-	-4.02±2.75	-
Azaguie_A	4.11 (0.0 - 19.46)	1910.3 (0.0 - 9063.3)	25.49 (0.0 - 128.41)	6859.7 (0.0 - 34429)	-1.20±1.64e-01	Resistant
Azaguie_B	2.22 (0.0 - 28.61)	1032.6 (0.0 - 13233)	10.8 (0.0 - 150.52)	2904.6 (0.0 - 40266)	-1.39±4.99e-01	Resistant
Binger	1.89 (0.0 - 19.98)	878.12 (0.0 - 9243.1)	4.85 (0.0 - 53.85)	1306.2 (0.0 - 14409)	-2.33±1.04	Resistant
Dabak	3.43 (0.0 - 11.25)	1596.2 (0.0 - 5290.8)	80.64 (0.0 - 314.19)	21699 (0.0 - 84376)	-6.96e-01±1.24e-01	Resistant
Toumodi	3.75 (0.0 - 21.61)	1743.1 (0.0 - 10036)	18.87 (0.0 - 114.00)	5076.6 (0.0 - 30542)	-1.36±1.59e-01	Resistant
Ferke_B	1.94e-01 (1.64e-01 - 0.22)	89.96 (42.97 - 136.95)	4.42e-01 (2.56e-01 - 0.63)	25.38 (0.0 - 75.551)	-2.66±4.66e-01	Resistant

LC₅₀: Concentration to kill 50% of the specimens in the sample; LC₉₀: Concentration to kill 90% of the specimens in the sample; CI: 95% confidence interval; RR: Ratio of Resistance; Houde: Sensitive strain of *Rhipicephalus geigy*.

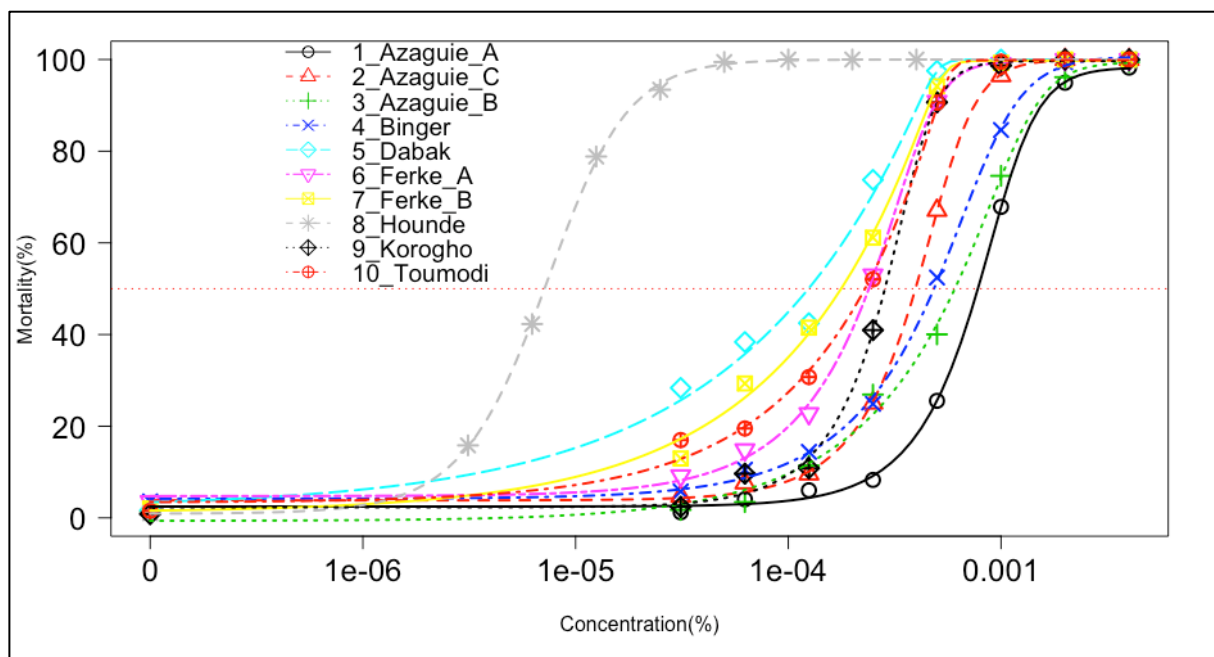


Figure 2: Dose-mortality curve with Amitraz.

Hounde (control), Korogho and Toumodi indicate samples from the corresponding locality (with the same names). Azaguie_A, Azaguie_B and Azaguie_C refer to samples from two herds located in Azaguie. Binger corresponds to samples from Bingerville. Dabak indicates samples from Dabakala whereas Ferke_A and Ferke_B refer to samples from Ferkessédougou.

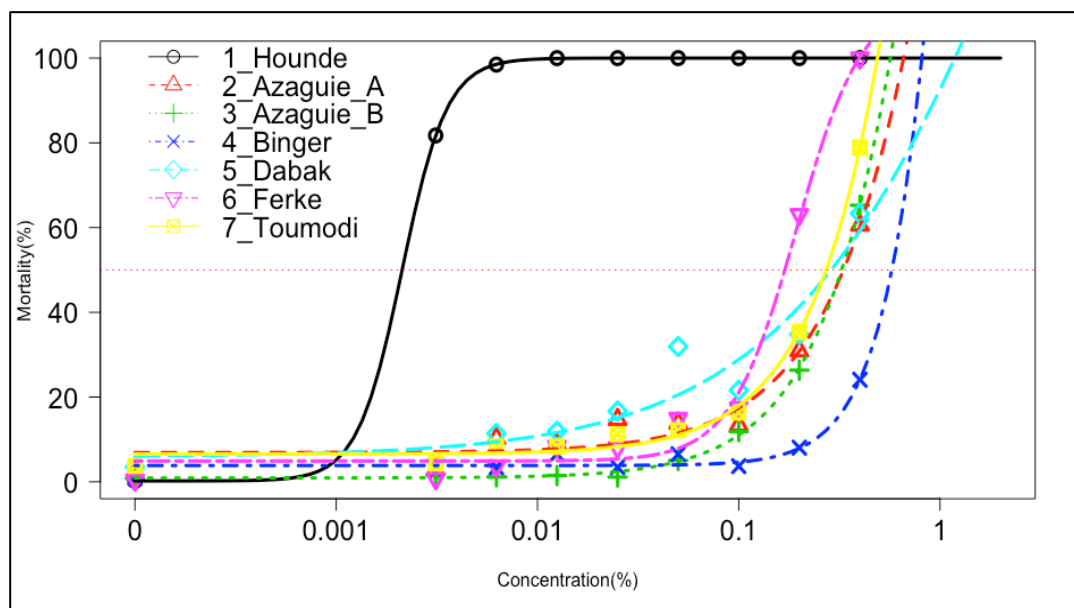


Figure 3: Dose-response curve with Deltamethrin.

Hounde (control) and Toumodi indicate samples from the corresponding locality (with the same names). Azaguie_A and Azaguie_B refer to samples from two different herds located in Azaguie. Binger corresponds to samples from Bingerville. Dabak indicates samples from Dabakala whereas Ferke refers to samples from Ferkessédougou.

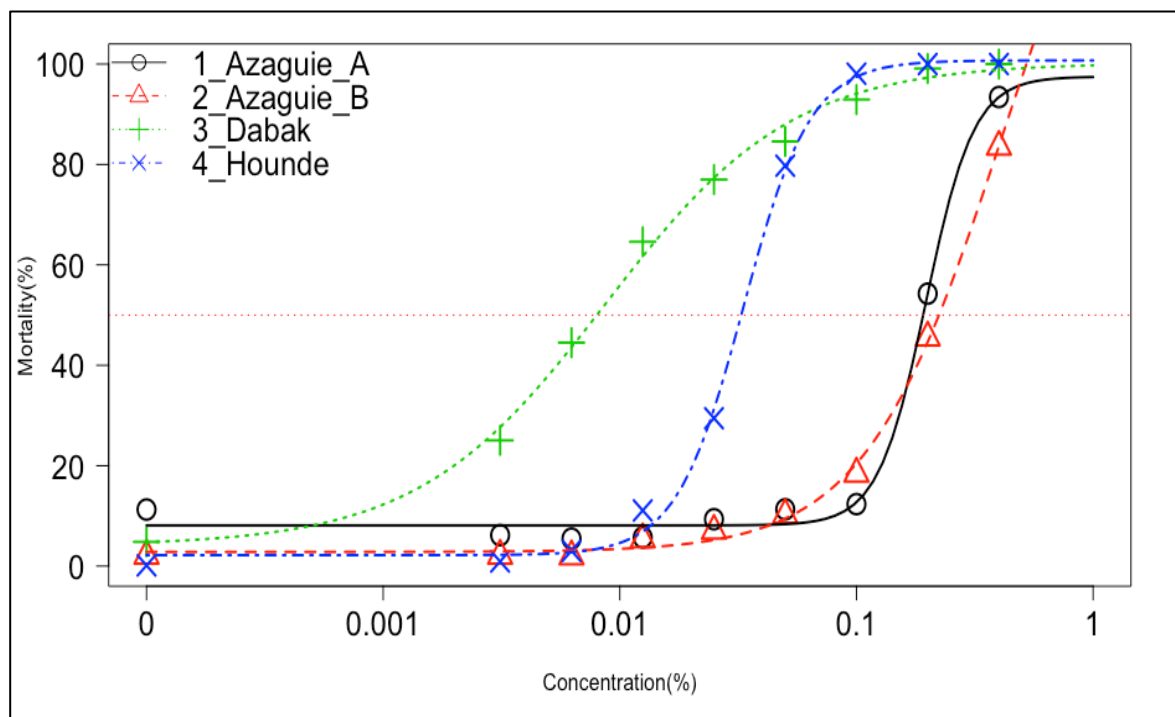


Figure 4: Dose-response curve with Alphacypermethrin.

Houde (control) indicates samples from the corresponding locality (with the same name). Azaguie_A and Azaguie_B refer to samples from two different herds located in Azaguie. Dabak indicates samples from Dabakala.

DISCUSSION

This study is the first evaluation of the resistance status of *R. microplus* population to acaricide products in several geographical areas of Côte d'Ivoire. This tick showed resistance to at least one of the three common acaricide molecules. Resistance of ticks, particularly the species *R. microplus*, has been reported in many countries around the world and in Africa, namely in Benin (Adehan et al., 2016) and Uganda (Vudriko et al., 2016).

Resistance to alphacypermethrin varied according to farm. These results shown similarity with those of a study conducted in Benin where resistance to acaricides was reported in all samples, except for the samples from Samiondji, which expressed susceptibility to alphacypermethrin (Adehan et al., 2016). Such pattern has also been highlighted in a study in Argentina (Cutullé et al., 2013).

Moreover, a study conducted at CIRDES on tick populations from Azaguie, which used the discriminating dose method, an

alternative qualitative method to the LPT method, showed resistance to deltamethrin and a susceptibility to amitraz at that time (Kandé, 2014). Selection rate, and progress in resistance to a given chemical depend on a number of factors, the most important being frequency of application of the same active ingredient across time. Six to seven years have passed since Kandé (2014) study, this long period could justify selection for resistance, and therefore the results found regarding amitraz. Otherwise, if the development of amitraz resistance in Australia might be relatively slow compared to the rapid spread of resistance to synthetic pyrethroids (Jonsson and Hope, 2007). In Mexico, many studies indicated a faster development of resistance to amitraz than that found in Australia, at 54.7% (Fernández-Salas et al., 2012) and up to 68.2% of the farms tested (Rodriguez-Vivas et al., 2013).

Thus, even if variation in resistance may be due to the tests used, it may also be related to the frequency or treatment practices that

differ with regions (Bianchi et al., 2003; Lovis, 2012). This could explain the results obtained in our study, which showed a variation in alphacypermethrin resistance on the farms.

The multi-resistance of *R. microplus* populations to amitraz and deltamethrin has also been reported in Martinique (Hamon, 2016) and New Caledonia (Hüe et al., 2016). To overcome these multiple resistances, various studies have evaluated the possibility of combining pyrethroids and amitraz to combine their acaricidal effects for optimal tick control (Li et al., 2007; Rodriguez-Vivas et al., 2013). In New Caledonia, the addition of amitraz to a deltamethrin solution made it possible to control deltamethrin-resistant tick populations (Barré et al., 2008). The success of mixtures is based on the hope that an animal is unlikely to carry resistant alleles for two or more acaricides with different modes of action. This strategy could be a special alternative to the use of a single acaricide in an integrated control programme.

These first results on the resistance of *R. microplus* in Côte d'Ivoire support the recommendation of alphacypermethrin use for tick control on some farms in the country. In order to assess the extent of *R. microplus* resistance to acaricides, it is necessary to extend this study to a larger number of farms and to other regions since this tick is currently disseminate throughout Côte d'Ivoire. Other acaricide products such as flumethrin, fipronil and other new treatments proposed locally and used in combination with traditional treatments should be test. This would enable the mapping of tick resistance to acaricides in order to provide advices to farmers for better controlling *R. microplus* tick in their herd, and allow for better control of this invasive species across Côte d'Ivoire.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

LYA, MB, AA and MK conceived and designed the study. LYA, MB, LSK and PRA conducted the study. AB, PKY and HA

analysed and interpreted the data. LYA wrote the first draft. MB, AB, PKY, HA, SK, AA and MK revised the manuscript. All authors read and approved the final version of the manuscript.

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