academicJournals

Vol. 11(14), pp. 1199-1208, 7 April, 2016
DOI: 10.5897/AJAR2015.10619
Article Number: 31AC14957918
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Acaricide resistance of *Rhipicephalus microplus* ticks in Benin

Safiou B. Adehan[1,3]*, Abel Biguezoton[2], Hassane Adakal[2], Marc N. Assogba[3], Sébastien Zoungrana[2], A. Michel Gbaguidi[1], Aretas Tonouhewa[3], Souleymane Kandé[5], Louis Achi[6,7], Hamade Kagone[4], Razaki Adehan[3], Guy A. Mensah[1], Reginald De Deken[8], Maxime Madder[8,9] and Souaïbou Farougou[3]

[1]National Institute of Agronomic Research/Agronomic Research Centre of Agonkanmey 2900 Cotonou/Benin Ministry of Agriculture, Breeding and Fishing, Benin.
[2]International Center of Research-Development on Breeding in Subhumid zone 01Box454 Bobo-Dioulasso01, Burkina-Faso.
[3]Polytecthnic School of Abomey-Calavi Research Unit in Biotechnology in Production and Animal Health O1 Box 2009 Cotonou, BÉNIN/Wecatic Project, Benin.
[4] Manager of Livestock, Fisheries and Aquaculture Programme-CORAF/WECARD, Dakar, Senegal.
[5]Rural Development Institute (IDR)/Polytechnic University of Bobo-Dioulasso, Burkina Faso.
[6]National Laboratory of Support to the Agricultural Development (LANADA) 04 BP 612 Abidjan 04, Côte d'Ivoire.
[7]Switzerland Center of Scientific Researches in Côte d'Ivoire (CSRS) 01 BP 1303 Abidjan 01, Côte d'Ivoire.
[8]Vector Biology Unit, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium.
[9]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa.

Received 5 November, 2015; Accepted 17 March, 2016

After the introduction of the invasive cattle tick *Rhipicephalus microplus* in West Africa in the last decade, farmers encounter ticks resistance to the use of acaricides in different region in Benin. In order to evaluate the level of resistance, an *in vitro* study was performed on five samples of *R.* (*Boophilus*) *microplus* collected from five farms in four of the eight agro-ecological zone of Benin. The districts concerned with the study in the agro-ecological zone were Houeyogbe (Kpinnou), Zangnanado (Samiondji), Tchaourou (Okpara), Gogounou (Fana) and Bassila (Manigri). A toxicological test, the Larval Packet Test (LPT) was performed in the laboratory of Biotechnology Research Unit of the Animal Production and Health (URBPSA) at the Polytechnic School of University of Abomey-Calavi in Benin with the susceptible, *Rhipicephalus geigyi* strain from Hounde in Burkina Faso. Three (3) acaricides commonly used by farmers in Benin to control ticks were evaluated: alpha-cypermethrin, deltamethrin and amitraz. The results showed that the resistance ratio at 50% (RR_{50} 95% CI) for the whole experiment varies from 1.96 to 338.5. Based on RR_{50} and RR_{90} values, only the population of Samiondji's state farm was susceptible to the alpha-cypermethrin with a resistance ratio RR_{50} = 1.64 (95% CI: 0.2 to 12.6), all the other resistance tests conducted on moderate or high resistance Bassila and Kpinnou appear to host the most resistant samples. Moreover, a certain high variability of dose response relationship has been noticed with amitraz on the base of the higher slope of the related curves.

Key words: Resistance ratio, distribution, *Rhipicephalus microplus*, acaricide, larval packet test (LPT), Benin.

INTRODUCTION

In Benin, recently conducted studies have revealed the presence of 10 ticks species in the northern part of the country and 11 species in central and southern part of the country (Farougou et al., 2007), including the recently introduced Asian cattle tick *Rhipicephalus microplus* (Madder et al., 2012). This tick species has a great ability for ecological adaptation and is suspected to be resistant to various conventional acaricides (Madder et al., 2012). However, the struggle against ticks and ticks borne pathogens transmitted not only depend on the effectiveness of acaricides used, but also and especially on the practice of the farmers.

A number of reports have described cases of resistance in *R. microplus* (also called blue ticks or cotton belt tick), because of a certain number of non-completely elucidated factors among which an inadequate posology of acaricide (Li et al., 2004; Ducornez et al., 2005). Organophosphoruses (OP) such as chlorpyriphos were the first acaricide molecules used in Argentina for which the resistance has been reported in the 1970s (Aguirre et al., 2000). Moreover, the first case of resistance to synthesis pyrethroids (SP) was reported in Argentina in 1996 (Caracostantogolo et al., 1996). Nearly 15 years later, the first resistance case to amitraz was identified (Cutullé et al., 2012).

The first cases of resistance of amitraz have been reported after ten and seven years of use respectively in Australia (Nolan, 1981) and Mexico (Soberanes et al., 2002; Andrew et al., 2004). In Mexico, *R. microplus* resistant ticks to organophosphate were documented in 1981. In 1993, the first cases of pyrethroid resistance were detected and in 2000 resistance to amitraz (Sanchez-Cespedes et al., 2002 Temeyer et al., 2004). Control of tick infestation for with acaricides began in the 1970s with diethylethion OP (ND Rhodiacide). From 1992 to the end of 1997, deltamethrin (ND Butox) was used but the tick population was declared resistant and was replaced by amitraz (ND Taktic).

Several studies have reported the resistance of *R. microplus* to acaricide in South Africa (Taylor and Oberem, 1995), Brazil (Martins et al., 1995; Furlong, 1999) and Colombia (Benavides et al., 2000). In the West African sub-region, all toxicology studies have been made on other tick species then *Rhipicephalus geigyi* and revealed no ticks resistance (Adakal et al., 2013b; Jongejan and Uilenberg, 2004; Kaljouw, 2008). Until now, no study has been conducted in Benin in order to determine the effectiveness of different acaricides used to control ticks, although *R. microplus* ticks are known to have developed resistance to many acaricides (Chevillon et al., 2007; Kumar et al., 2012; Lovis et al., 2013).

In view of this, there is a strong need to evaluate the level of resistance of *R. microplus* to "… commonly used acaricides by farmers in Benin where inefficiency of acaricides is reported (Madder et al., 2012). Infestations with this exotic tick constitute a serious threat to livestock production and health in West africa. The invasive nature of this tick and especially its ability displace of other native ticks species of the same genus *Rhipicephalus annulatus, R. geigyi* or *Rhipicephalus decoloratus* has been documented recently (Madder et al., 2012). This study will rehabilitate programs against these parasites based on the data obtained. Indeed, it is recognized in the world that *Bos indicus* cattle type is more resistant to ectoparasites than *Bos taurus* cattle (Mattioli et al., 1993; Bianchin et al., 2007).

The present study aims to evaluate the resistance of the cattle tick *R. microplus* along a north-south Region of Benin.

MATERIALS AND METHODS

Study area and collection of ticks

R. microplus males and engorged females were collected from cattle at the state farms of Kpinnou (Houeyogbe), Opkara (Tchaourou), Samiondji (Zangnanado) and at two private farms in Fanan (Gogounou) and Manigri (Bassila) between October and December, 2013, a period of high tick abundance (Farougou et al., 2007). Ten cattle between 2 and 3 years of age and visibly infested with ticks, were selected on each of the farms. The animals received no acaricide treatment, at least three months prior to the day of collection. Ticks were collected on different species of cattle of Girolando (Kpinnou), Lagunaire (Samiondji), Borgou (Okpara, Bassila and Gogounou). The collection from each animal was kept in a separate labeled tube with perforated lid. The animal number, date of collection, name of farm, the village, the town and the county was recorded on the label. All samples were geo-referenced using a Garmin Xtrex.

Tick identification and rearing of larvae

The identification of ticks and resistance tests were performed in the Laboratory of Biotechnology Research Unit of the Animal Production and Health (the URBPSA) at the Polytechnic School of Abomey Calavi (PSAC) of University of Abomey in Benin. For the purposes of the acaricide resistance test, 50 engorged females ticks collect on at least ten animals (maximum eight of one animal) identified as *R. microplus* for each of the five farms were placed in empty vials perforated medium fine mesh and placed in an incubator at a temperature between 27 and 29°C with a relative humidity between 85 and 95% (Lovis et al., 2013).

After oviposition, the eggs were collected during 3 to 4 days post-laying, weighed and placed in tubes covered with fine mesh fabric at a rate of 0.5 g per tube. The larvae used in these resistance tests

*Corresponding author. E-mail: adehankarim@yahoo.fr. Tel: + 22996785061.

were aged from 14 to 21 days (Lovis et al., 2013).

Larval packet test pocket preparation

The Larval Packet Test (LPT) standard (FAO, 1984) was used to evaluate the acaricide resistance of ticks to acaricides. The filter paper Whatman used to manufacture packets for larvae was performed at CIRDES. Three acaricides at variable purity (Table 1), supplied by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) were tested. This is deltamethrin, alpha-cypermethrin and amitraz, all in powder form. A serial dilution of each acaricide was prepared using a solvent, composed of 1 volume of olive oil mixed with 2 volumes of trichlorethylene (Miller et al., 2002). Seven different concentrations were prepared for each acaricide test (Table 1) with two filter papers (8.5 × 7.5 cm) by concentration. The assays were performed in two replicates. A volume of 0.67 ml of each dilution was applied using a micropipette on each of two Whatman paper filters (Cat. No. 3001 917). The papers were then dried under a hood, with gas aspiration to outside for 2 h to allow the evaporation of trichlorethylene. The dried papers were then wrapped in aluminum foil by concentration and kept refrigerated at 4°C. For each test, two whatman filter papers impregnated with acaricide were used by concentration. The amount of M pure acaracide to be used for the preparation of the stock solution is obtained by the formula: M= X / (% of purity) × (20/3), where X = % of the highest concentration, final volume = 20 and 3 = a constant.

Reference tick strain

In the absence of a susceptible references strain of *R. microplus*, the level of resistance of the collected ticks was compared with a susceptible strain of *R. geigyi.* This strain was collected on Baoule cattle breed, in Hounde, a town in the province of Tuyin Hauts-Basins in south western of Burkina Faso. It was maintained at CIRDES for twelve generations (four years), fed on the same cattle breed and did not show any signs of resistance (Adakal et al., 2013a).

Execution of the larval packet test

The aluminum foils containing the impregnated papers were opened using forceps, each paper was folded in two. Each folded acaricide paper was retained by two plastic clasps to form an open pocket on one side. The pocket receives using a brush number N°4, approximately 100 tick larvae through the open side of the folded paper. This side was then closed with a third clasp. After doing this for all concentrations, all envelopes were put into a tray and placed in a heat chamber for 24 hours at a temperature of 28±1°C with a humidity of 90 ± 5% (Lovis et al., 2011). Each envelope was marked with pencil by the corresponding concentration, the name of the acaricide used and date of preparation. After 24 h of incubation, the individual packages are removed from the heat chamber to control larval mortality in each envelope.

The concentrations used in our tests ranged from 0.0156 to 4%, depending on each lethal concentration known of acaricide (Adakal et al., 2013a; Lovis et al., 2013).

Statistical analysis

Non-linear regression analyses of dose-mortality data were performed in R (version 2.15.3) (Ritz and Streibig, 2005) using the drc (Dose-Response Curves) package (version 2.3-96), specific for modelling dose-response curves (Ritz and Streibig, 2005), As age of larvae considered was different from one acaricide to another, they were considered as covariance in the modelling as well. Three functions: four-Log-logistic model, four and three Weibull models were tested in order to choose the one giving the lowest residual deviance. To model the data using the Dose Regression Mortality (DRM) command, bottom and top values locked at 0 and 100, respectively. LC_{50} and LC_{99} values and their 95% confidence intervals (95% CI) were estimated using the effective dose (ED) command and the delta option for the interval parameter. The difference between LC_{50} estimated was designated as significant if their 95% CI did not overlap. The resistance ratios (RR) of *R. microplus* at Gogounou, Kpinnou, Samiondji, Okpara and Bassila were computed relatively to the susceptible reference strain tick *R. geigyi* Hounde 2005. The plots of the model were performed to well evaluate trends that each model presented.

Resistance ratios and their CI at 95% were calculated, so were the slopes and intercepts of the regression line (Robertson and Preisler, 1992). Differences are significant when the number 1 is excluded from the CI of resistance ratio (Ducornez et al., 2005). According to Jonsson and Hope (2007), a tick population is said to be sensitive to an acaricide when RR < 4; moderately resistant if 4 <RR <10 and super-strong when RR> 10.

RESULTS

General trend

Four-parameters Log-logistic is the best model for the present analysis (residual deviance=0.60). Lethal concentrations 50 and 90 (LC_{50} and LC_{90}) and their 95% CI are reported in Tables 1, 2 and 3 for the three acaricides tested: deltamethrin, alpha-cypermethrin and amitraz respectively. The resistance ratio 50 and 90 (RR_{50} and RR_{90}) and their related 95% CI are also reported in the same tables for the three acaricides tested. These values made it possible to carry out a geographic distribution card of the *R. microplus* resistance to usual acaricides in Benin (Figure 1). It appears that RR_{50} CI are narrower than that of RR_{90} for alpha-cypermethrin and deltamethrin; at the opposite, amitraz does not follow the same trend. The deltamethrin dose responsive curve of Gogounou and Samiondji samples show a greater slope than that of the reference, the same situation occurs with alpha-cypermethrin dose responsive curve for Gogounou and Okpara sample and also with amitraz dose responsive curve for Gogounou, Bassila and Okpara samples.

For each of the acaricides used, no mortality was recorded at the lowest concentrations in the susceptible reference sample. With the susceptible reference sample, 100% mortality was recorded at the highest concentration for each of the three acaricides used. The number 1 is excluded from the CI of RR_{50} and RR_{90} for all field samples, which lead to a significant difference between any of the field sample and the Hounde strain (2005) at 95% CI.

Resistance to deltamethrin

Hounde's responsive curve occupy a backward position

Table 1. Different sets of dilution applied to "pure" tested acaricides.

Acaricide (purity percentage)	Chemical family	Strains (Latitude, Longitude)	Dilution series (%)
Alpha-cypermethrin (95%)	Synthesis Pyrethroids	Kpinnou (N6.56828, E1.78623)	
Deltamethrin (99.5%)	Synthesis Pyrethroids	Samiondji (N7.41667, E2.36667) Gogounou (N10.73843, E2.92359)	0 - 0.0078 - 0.0156 - 0.0312 - 0.0625 - 0.125 - 0.25 - 0.5 - 1 - 2 - 4
Amitraz (98%)	Amidines	Okpara (N9.30501, E2.73148) Bassila (N8.94135, E1.77063)	
Alpha-cypermethrin (95%)	Synthesis Pyrethroids	Hounde (N11.48333, W3.51667)	0.0312 - 0.0625 - 0.125 - 0.25 - 0.5 - 1
Deltamethrin (99.5%)	Synthesis Pyrethroids		0.0281-0.0562- 0.112- 0.225 - 0.45 - 0.9
Amitraz (98%)	Amidines		0.0256-0.0512 - 0.102 - 0.205 - 0.41 - 0.82

Hounde: sensitive reference strain; Kpinnou, Samiondji, Gogounou, Okpara and Bassila: field strain; AI: active ingredient

Table 2. Lethal Concentration (LC_{50} and LC_{90}) and Deltamethrin Resistances ratios (RR_{50} and RR_{90}).

Sites	Deltamethrin				Slopes
	LC_{50} (CI)	LC_{90} (CI)	RR_{50}	RR_{90}	
Hounde	0.02 (0.016-0.023)	0.04 (0.029-0.054)	-	-	2.419±0.072
Gogounou	0.87 (0.80-0.94)	1.78 (1.44-2.12)	54.42 (51.76-56.89)	44.94 (38.79-50.39)	3.065±0.366
Okpara	0.85 (0.78-0.93)	1.70 (1.38-2.03)	53.59 (50.74-56.24)	43.01 (37.00-48.33)	1.794±0.057
Bassila	2.27 (1.86-2.68)	13.05 (6.94-19.17)	142.09 (120.32-162.42)	329.64 (186.61-406.20)	1.256±0.135
Samiondji	0.60 (0.55-0.66)	1.27 (0.97-1.56)	37.70 (35.67-39.88)	31.95 (26.19-37.05)	3.191±0.407
Kpinnou	2.29 (1.86-2.72)	13.41 (6.93-19.88)	143.34 (120.64-164.53)	338.50 (186.39-473.09)	1.243±0.136

LC (%): Lethal concentration at 95%; CI: Confidence intervals at 95%; RR: Resistance ratios

in comparison with all the field sample curves (Figure 2A). Gogounou and Okpara curves in one hand and Bassila and Kpinnou curves in second hand appear conflicting (Figure 2A). This pattern tends to be confirmed by their LCs (0.87/0.85 and 1.78/1.70) and RRs (54.42/53.59 and 44.94/43.01) which are very closed (Table 2).

The LC_{50} and LC_{90} of deltamethrin for Hounde strain are respectively 0.02% (0.016 to 0.024) and 0.04% (0.029 to 0.054) at 95% CI. They are weaker compared to those of the field samples. The higher LC_{50} and LC_{90} among field samples are those of Kpinnou sample followed by Bassila, Gogounou and Okpara samples. Those of Samiondji (0.65 and 1.27%) are the smallest of all field samples.

All of the field samples have their resistance ratios above 10; except that of Okpara which is greater than 4. This leads to admit that Okpara sample shows moderate resistance to deltamethrin whereas the other field samples are highly resistant to all concentrations of deltamethrin (Table 2). Moreover, there is no emerging resistance to deltamethrin but only established resistance.

Table 3. Lethal Concentration (LC50 and LC90) and Alpha-cypermethrin Resistances ratios (RR50 and RR90)

Sites	Alpha-cyperméthrine				Slopes
	LC_{50} (CI)	LC_{90} (CI)	RR_{50}	RR_{90}	
Hounde	0.03 (0.03-0.04)	0.09 (0.07-0.12)	-	-	2.191±0.266
Gogounou	1.04 (1.02-1.07)	2.10 (1.98-2.22)	30.42 (27.20-34.75)	22.50 (19.11-28.08)	3.133±0.111
Okpara	1.04 (1.01-1.06)	02.08 (1.97-2.20)	30.33 (27.11-34.65)	22.31 (18.94-27.84)	3.159±0.112
Bassila	2.29 (1.86-2.73)	13.36 (6.89-19.82)	66.96 (63.64-69.43)	143.0 (97.61-170.59)	1.247±0.137
Samiondji	0.07(0.05-0.09)	0.21(0.08-0.34)	01.96 (01.68-02.17)	02.28 (01.18-02.96)	1.902±0.445
Kpinnou	1.24 (1.18-1.30)	3.38 (3.01-3.77)	36.20 (33.23-40.19)	36.24 (32.40-42.55)	2.189±0.110

LC (%): Lethal concentration at 95%; CI: Confidence intervals at 95%; RR: Resistance ratios.

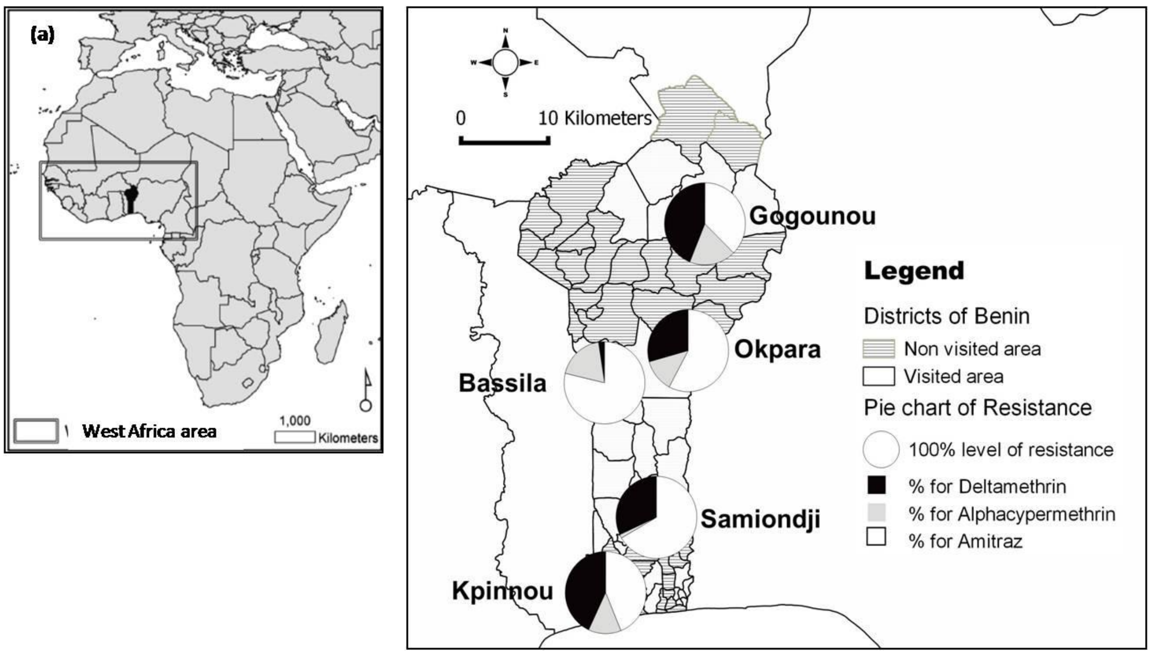

Figure 1. (a) The geographical location of Benin in West Africa and (b) the distribution map of the resistance of *R. microplus* resistance in Benin.

Kpinnou strain is characterized by exceptional very high values of RR_{50} and RR_{90}, respectively 143.34 and 338.50.

Resistance to alpha-cypermethrin

Hounde and Samiondji curves occupy the backward positions (Figure 2B) with Samiondji slightly behind Hounde; forwardest position is that of Bassila curve. Gogounou and Okpara curves appear conflicting, slightly behind and very close to that of Kpinnou (Figure 2B).

Lowest LC_{50} and LC_{90} are those of Hounde (0.0.3 and 0.09) and Samiondji (0.07 and 0.21) whereas the highest are those of Bassila (2.29 and 13.26). All the RRs values related to the field samples are above 10 except that of Samiondji strain which is less than 4. Consequently, Samiondji strain is susceptible whereas the other strains are resistant. Moreover, for resistant strains, there is no emerging resistance, but only established resistance. Bassila shows the highest level of resistance to alpha-cypermethrin with RR_{50} and RR_{90}, respectively equal 66.96 and 143.

Resistance to amitraz

Hounde and Samiondji curves are close although Hounde curve is slightly behind the second one (Figure 2C). The forwardest curve is that of Kpinnou. Okpara and Bassila curves occupy a medium position with Gogounou curve that is slightly behind but more forwardly open than the two others (Figure 2C). Curves slopes of

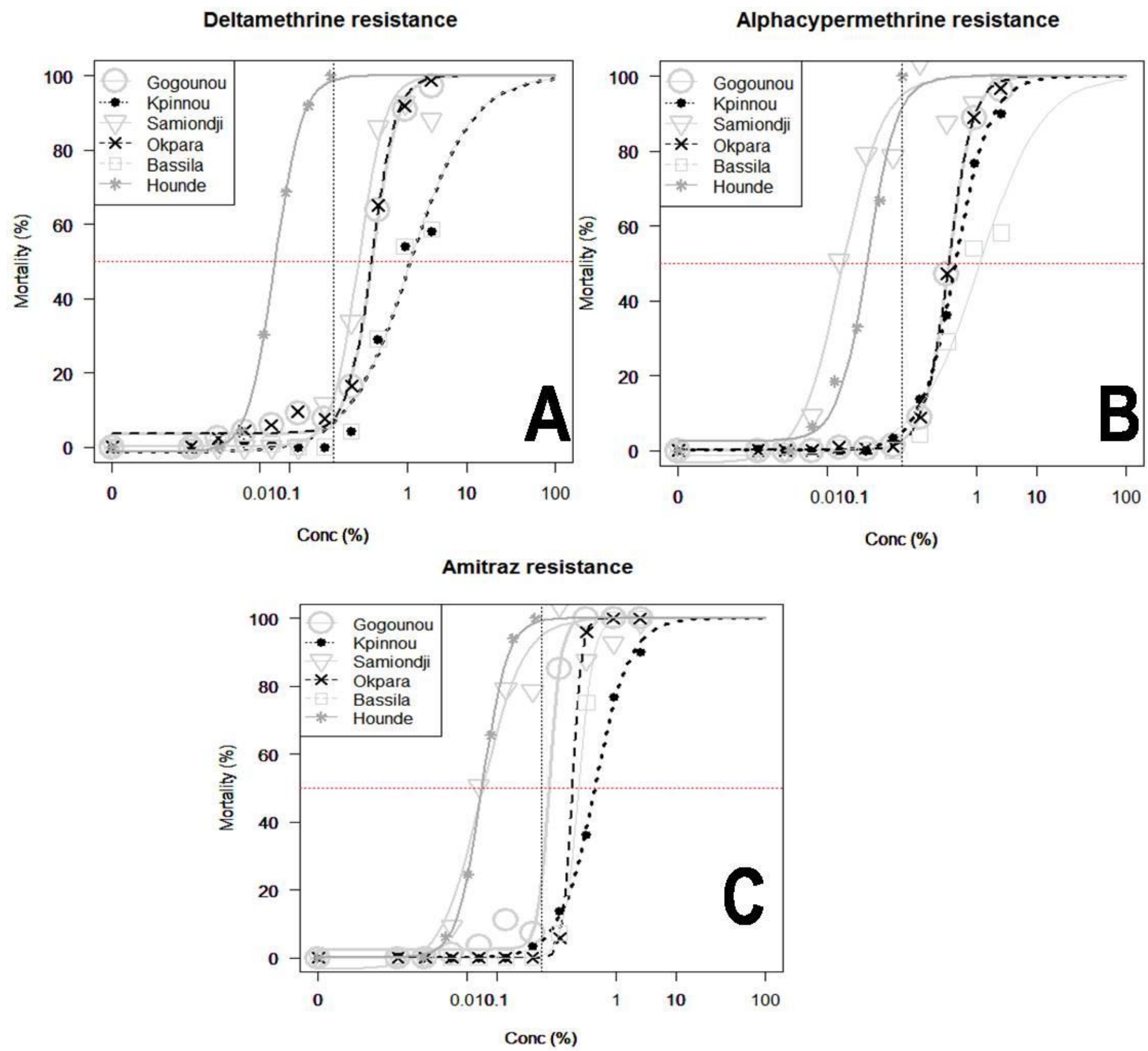

Figure 2. Dose-response curves of five Benin field populations in comparison to the susceptible reference strain of Houndé when tested with three acaricides (The gray dotted horizontal lines indicate 50 and 90 % mortalities)

field samples are among the highest of response curves; up to three or four times greater than that of reference strain.

The lowest values of LC are those of Hounde strain (0.02 and 0.04) and Samiondji sample (0.07 and 0.21) whereas the highest is that of Kpinnou (1.24 and 3.39).

All the RRs values related to the field samples are above 10 except that of Samiondji strain which is greater than 4 and less than 10. Consequently, Samiondji strain is moderately resistant to amitraz whereas the other strains are highly resistant to this acaracide. All the cases of resistance are established resistance; there is neither emerging resistance nor susceptibility. Kpinnou sample expresses the higher level of resistance with resistance ratio about 78.5 and 92.89, respectively for RR_{50} and $RR_{90;}$ Bassila sample shows the second resistance ability with RR_{50} and RR_{90} values about 51.38 and 33.7.

Relatively to amitraz, RR_{50} and RR_{90} values are greater than 10. RR_{50} values are very high and comprehended between 4.25 (03.23 - 05.21) and 78.53 (77.16 - 79.82), whereas RR_{90} values vary between 05.84 (02.43 - 08.89) and 92.89 (87.82 - 97.37). Resistance to amitraz for all the samples was concluded. In details, apart from Samiondji strain that shows a moderate resistance, all the other field samples show high resistance to amitraz. The little succeptibility of Samiondji sample to amitraz appear in the graph through the proximity between Samiodji and Hounde curve. All the over curves are forwardly open from the two last (Figure 2C). Moreover, in general response, curves to Amitaz are more

Table 4. Lethal Concentration (LC_{50} and LC_{90}) and Amitraz Resistances ratios (RR_{50} and RR_{90}).

Sites	Amitraz				Slopes
	LC_{50} (CI)	LC_{90} (CI)	RR_{50}	RR_{90}	
Hounde	0.02 (0.02-0.02)	0.04 (0.03-0.04)	-	-	2.628±0.088
Gogounou	0.38 (0.36-0.42)	0.54 (0.50-0.57)	24.58 (23.60-25.49)	14.77 (14.70-14.83)	6.724±0.847
Okpara	0.69 (0.69-0.70)	0.90 (0.89-0.90)	43.82 (42.54-45.19)	24.57 (23.31-26.01)	8.524±0.055
Bassila	0.81 (0.77-0.86)	1.23 (1.14-1.32)	51.36 (50.26-52.38)	33.70 (33.27-34.08)	5.297±0.538
Samiondji	0.07 (0.05-0.09)	0.21 (0.08-0.34)	04.25 (03.23-05.21)	05.84 (02.43-08.89)	1.902±0.445
Kpinnou	1.24 (1.18-1.30)	3.39 (3.01-3.77)	78.53 (77.16-79.82)	92.89 (87.82-97.37)	2.189±0.110

LC (%): Lethal concentration at 95%; CI: Confidence intervals at 95%; RR: Resistance ratios.

distinguishable one from the other comparatively to those of the other acaracides.

DISCUSSION

Confirmed tick resistance to acaracide in Benin

Results of study make find out resistance among most of the ticks field samples. Apart from the Samiondji sample, which is still susceptible to alpha-cypermethrin, all the other samples showed moderate resistance ($4<RR_{50}<10$) to strong resistance ($RR_{50} > 10$) to all three acaricides (Tables 2, 3 and 4). Similar cases of resistance have been reported by Andreotti (2007); especially, resistance to pyrethroids (SP), namely alpha-cypermethrin and cypermethrin of several samples of *R. microplus* collected from farms in the state of Matto Grosso do soul Brazil. Following recent results obtained by Lovis et al. (2013), the resistance (moderate or high) to deltamethrin and alpha-cypermethrin of Gogounou, Okpara, Bassila and Kpinnou sample and also resistance to deltamethrine of Samiondji strain can be considered as fully established. Since their RR_{50} and RR_{90} follow the same trend (in comparison with the cut off values that are 4 and 10). Therefore, no emerging resistance is noticeable; indeed, emerging resistance is admitted when RR_{90} and RR_{50} are respectively higher and lower than 4 with the slope of the field population small than that of the reference as published by FAO (2004) cited by Lovis et al. (2013).

Similar results, related to established resistance has been found for the *R. microplus* strains ST27, ST26, ST25, ST22 in Argentina and other strains in South Africa and also Australia with Fluomethrin and Cypermethrin which are synthetic pyrethroids (Lovis et al., 2013). Miller et al. (2003) also mentioned resistance to pyrethroids of a strain from Texas.

Overall, some countries concerned with systematic treatments based on pyrethroids and where resistance studies has been performed encountered resistance to pyrethroids (Leite, 1988; Laranja et al., 1989; Rodriguez-Vivas et al., 2006; Rodriguez-Vivas et al., 2007; Chevillon et al., 2007; Kearney, 2011). Considering the levels of resistance, examples of deltamethrin resistance are reported in New-Caledonia (Chevillon, 2007) with RRs values smaller than 30; these levels of resistance are lower than those of Samiondji and Gogounou and especially at least five times lower and up to ten times lower than that of Kpinnou (RR_{50}=143.34 and RR_{90}=338.5). It leads to the conclusion that levels of resistance in Benin reach high levels compared with that of the main countries where resistance studies has been performed.

Gogounou and Okpara host the most homogeneous samples

Although Gogounou sample expresses high level resistance to all acaricides; the slopes of dose responsive curves of Gogounou sample are steeper than that of reference; this is an indicator of either a certain high susceptibility of Gogounou sample or a very significant dose-mortality relationship. In fact, small slope make it difficult to discriminate between susceptible and resistant individuals (Miller et al., 2002). An extreme low slope should be the sign of a clearly resistant sample which consist of homogeneous resistant individuals. At the opposite, the steeper the slope is, the higher the response from the sample is; that is a significant and noticeable increment in tick mortality according to increasing doses. An extreme high slope should be the sign of a clearly susceptible sample which consist of homogeneous susceptible individuals. Since LC_{50} and LC_{90} related to Gogounou are higher than that of the reference and on the bases of RRs values, Gogounou sample which is concerned with an established resistance to the three acaricides, might consist of resistant individuals that should be less resistant and more homogeneous than other field samples, with regard to the resistance ability. Indeed, an opposite situation is that of Lovis et al. (2013) who find out emerging resistance to flumethrin associated with an heterogeneous population, on the base of smaller slope with RR90 greater than RR50. Okpara appears to be the second point of homogenous sample.

Kpinnou and Bassila respectively as first dissemination point and abundance point of ticks host most resistant tick samples

Moreover, the gap between the resistance levels in Kpinnou and those of the other sites in Benin might be interpreted as an increase of resistance since Kpinnou is the first introduction point in Benin (Madder et al., 2012). Increase of resistance level for Kpinnou sample is particularly spectacular since *R. microplus* control in Kpinnou is at least fourteen years recent than that of New-Caledonia (Chevillon et al., 2007).

Bassila that is recognized as a point of abundance of ticks in Benin (De Clercq et al., 2013; De Clercq et al., 2012), appears to be the second point of relatively high level of resistance behind Kpinnou. This point of abundance of ticks is a grouping point of transhumance. However, it is curious how this grouping point of transhumance show no emerging resistance and instead, an evenly established high level of resistance to deltamethrin and alpha-cypermethrin. Therefore, Bassila sample does not show large phenotypic diversity to deltamethrin and alpha-cypermethrin. The situation should be caused by a rapid regeneration and high number of reproductive cycles of this parasite under favourable environment conditions or continuous deltamethrin and alpha-cypermethrin tick control actions by farmers. This explanation might be the same for the high level resistance of Kpinnou strain relatively to Pyrethroids and it is likely that long time exposure to the related acaricide since the first introduction is of importance. At this step, it raised the necessity of assessing the mechanism of resistance to well understand the comparative evolution of Bassila and Kpinnou samples respectively as transhumance grouping point and first introduction or dissemination point. Many studies used synergists (Chevillon et al., 2007) to master the mechanism, further related experiments should be achieve for Benin sample. Comparatively to New caledonian resistant strain, the samples that shows punctual relatively high level of resistance like Gogounou sample which expressed resistance to delthamethrin and alpha-cypermethrin and Okpara sample that expressed resistance to alpha-cypermethrin, look more resistant. This resistance of Benin samples might not be explained by long time exposure but mainly as consequences of misuses of acaracides (Achukwi et al., 2001). In fact, the repeated use of the same acaricide by farmers in a given area eventually led to serious problems; resistance ticks to some of these acaricides has been reported (Musonge and Tanya, 1987); moreover, it improves fast selection of resistance monotropic and monophasic short-cycle species that are much more likely to develop resistance mechanisms. Indeed, several authors have reported that when generations succeed rapidly for a given species of parasite, the selection of resistant subpopulations is easy; particularly evident in cattle tick *R. microplus* whose parasitic phase on the host is twenty-one days (Barré and Uilenberg, 2010; Guerrero et al., 2012).

Samiondji host most susceptible samples

Taking into account the susceptibility of Samiondji sample to alpha-cypermethrin and the moderate resistance of the sample to amitraz, Samiondji sample shows the lower resistance ability. The susceptibility of Samiondji sample is also confirmed by the highest slope of deltamethrin dose responsive curve behind the reference strain. However, the change in level of resistance of Samiondji sample according to the acaricide express variation in the mechanisms of tick resistance in one hand and also in the acaricide mode of action in the second hand. This issue is particularly interesting if they take into account the change in level of resistance of Samiondji according to the two different pyrethrenoids (alpha-cypermetgrin and deltamethrin). Assessing this particular change in the mechanism of resistance or susceptibility to the two pyrethrenoids will help understand the base of mechanisms.

Particular trend of amitraz dose-response relationship

Since the slope of amitraz dose-responsive curves are among the highest of all the dose-responsive curves, it can be conclude that the amitraz dose responsive relationship is particularly significant with LPT. Samiondji, Bassila, and Okpara samples express the same trend than that of the results obtained by Lovis et al. (2013) with LPT. Opposite trend to those results have been found with amitraz by Kemp et al. (1998) and reported by Mendes et al. (2013). In fact, these related authors point out the lower efficiency of LPT test with Anitraz in comparison with organophosphat, pyrethroid and chlorinated hydrocarbon. Therefore, Samiondji, Bassila and Okpara samples show some specificity in comparison with the strains mentioned by Kemp et al. (1998), Miller et al. (2002) and Mendes et al. (2013). Another particularity is the lower size of RR_{90} confidence interval in comparison with RR_{50} confidence interval for the three samples of Benin. Again, this trend is the same than the results reported by Lovis et al. (2013). In fact Lovis et al. (2013) agues inadequacy of RR_{90} to identify amitraz resistance with LTT test. All in one, the differences observed with amitraz should be object of more investigation; especially to access effect of strain and acaricide interactions on dose responsive curves and also on the efficiency of amitraz resistance assessment with the different tests.

Conclusion

The results showed that most of the ticks populations collected were resistant to alpha-cypermethrin, deltamethrin and amitraz, apart from the sample of Samiondji which is susceptible to alpha-cypermethrin. However, the level of resistance vary according to the sample and the accaricide in such a way that the real challenge remains to get more knowledge about the history and process of the resistance, particularly through deeper comparisons between Bassila and Kpinnou strains. Also, the particular sensitivity of dose response relationship demand more exploration.

All in one, in order to solve resistance phenomenon, there is a great need to consider other alternatives for an efficient and safe control of ticks especially for *R. microplus* currently invading the West African sub-region and Benin. Some molecules of plant extracts have been shown to be effective organs on *R. microplus* and should be further tested. Also, molecules of the latest generation with growth regulators like fluazuron or Spinosyns (spinosad) are little known in the West African sub-region and against which *R. microplus* have not yet developed resistance should receive the same attention.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to thank CIRDES (International Center of Reseach-Development on Breeding in Subhumid Zone), EPAC/UAC (Polytechnic School of Abomey-Calavi University) and the WECATIC (Assessment of emerging livestock ticks and tick-borne disease threats and integrated control strategies in West and Central Africa) Project for the financial support of this research as part of a Ph.D program offered, Nestor Ahomadegbe, herdsman at the state farm in Kpinnou is thanked for their assistance during the ticks collect work.

REFERENCES

Achukwi MD, Tanya VN, Messine O, Njongmeta LM (2001). Etude comparative de l'infestation des bovins Namchi (Bos taurus) et Goudali de Ngaoundéré (Bos indicus) par la tique adulte Amblyomma variegatum= Comparative study of the infestation of Namchi (Bos taurus) and Ngaoundere Gudali (Bos indicus) cattle by Amblyomma variegatum adult ticks= Estudio comparativo de la infestacion de bovinos Namchi Bos taurus) y Gudali de Ngaoundere (Bos indicus) mediante la garrapata adulta Amblyomma variegatum. Rev. Elev. Med. Vet. Pays Trop. 54(1).

Adakal H, Biguezoton A, Zoungrana S, Courtin F, De Clercq EM, Madder M (2013a). Alarming spread of the Asian cattle tick *Rhipicephalus microplus* in West Africa--another three countries are affected: Burkina Faso, Mali and Togo. Exp. Appl. Acarol. 61(3):383.

Adakal H, Stachurski F, Chevillon C (2013b). Tick control practices in Burkina Faso and acaricide resistance survey in *Rhipicephalus (Boophilus) geigyi* (Acari: Ixodidae). Exp. Appl. Acarol. 59:483-491.

Aguirre DH, Vinabal AE, Salatin AO, Cafrune MM, Volpogni MM, Mangold AJ, Guglielmone AA (2000). Susceptibility to two pyrethroids in *Boophilus microplus* (Acari: *Ixodidae*) populations of northwest Argentina: Preliminary results. Vet. Parasitol. 88(3):329-334.

Andreotti R (2007). A synthetic BmTI n-terminal fragment as antigen in bovine immunoprotection against the tick *Boophilus microplus* in a pen trial. Exp. Parasitol. 116(1):66-70.

Andrew Yl, Davey BR, Miller J, George J (2004). Detection and Characterization of Amitraz Resistance in the Southern Cattle Tick, *Boophilus microplus* (Acari: *Ixodidae*). J. Med. Entomol. 41:193-200.

Barré N, Uilenberg G (2010). Propagation de parasites transportés avec leurs hôtes: Cas exemplaires de deux espèces de tiques du bétail. Rev. Sci. Technol. Off Int. Epiz 29:135-147.

Benavides E, Rodriguez JL, Romero A (2000). Isolation and partial characterization of the Montecitos strain of *Boophilus microplus* (Canestrini, 1877) multiresistant to different acaricides. Ann. N. Y. Acad. Sci. 916(1):668-671.

Bianchin I, Catto JB, Kichel AN, Torres RAA, Honer MR (2007). The effect of the control of endo- and ectoparasites on weight gains in crossbred cattle (Bos taurus Taurus × Bos taurus indicus) in the central region of Brazil. Trop. Anim. Health Prod. 39:287-296.

Caracostantógolo J, Muñoz CME, Eddi C, Ambrústolo RR, Bulman GM, Marangunich L (1996). Primera determinación en la República Argentina de una población de *Boophilus microplus* (Can.) resistente al piretroide sintético alfacipermetrina caracterizada mediante pruebas preliminares. Vet. Arg. 13:575-582.

Chevillon C, Ducornez S, Meeu T, Brou BK, Gai H, Delathiere JM, Barre B (2007). Accumulation of acaricide resistance mechanisms in *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) populations from New Caledonia Island. 26. Vet. Parasitol. 147:276-288.

Cutullé C, Lovis L, d'Agostino BI, Balbiani GG, Morici G, Citrioni D, Reggi J, Caracostantógolo JL (2012). *In vitro* diagnosis of the first case of amitraz resistance in *Rhipicephalus microplus* in Santo Tomé (Corrientes). In Santo Tomé (Corrientes), Argentina. Vet. Parasitol. 192(1):296-300.

De Clercq EM, Estrada-Peña A, Adehan S, Madder M, Vanwambeke SO (2013). An update on distribution models for *Rhipicephalus microplus* in West Africa. Geospat. Health 8(1):301-308.

De Clercq EM, Vanwambeke SO, Sungirai M, Adehan S, Lokossou R, Madder M (2012). Geographic distribution of the invasive cattle tick *Rhipicephalus microplus*, a country-wide survey in Benin 15. Exp. Appl. Acarol. 58:441-452.

Ducornez S, Barre N, Miller R, De Garine-Wichatitsky M (2005). Diagnosis of amitraz resistance in *Boophilus microplus* in New Caledonia with the modified Larval Packet Test. Vet. Parasitol. 130:285-292.

FAO (2004). Resistance management and integrated parasite control in ruminants: guidelines. Module 1. Ticks: Acaricide Resistance: Diagnosis, Management and Prevention. FAO, Rome, Italy pp. 25–77.

FAO (1984).Ticks and tick-borne disease control. A practical field manual. Vol. I. FAO Rome pp. 247–248.

Farougou S, Kpodekon M, Tassou AW (2007). Abondance saisonnière des tiques parasites des bovins dans la zone soudanienne du Bénin: cas des départements du Borgou et de l'Alibori. Rev. Afr. Santé Prod. Anim. 5:61-67.

Furlong J (1999). Diagnosis of the susceptibility of the cattle tick, *Boophilus microplus*, to acaricides in Minas Gerais State, Brazil. In: IV Seminario internacional de parasitologia animal-Control de la resistancia en garrapatas y moscas de importancia veterinaria y enfermededades que transmiten, Puerto Vallarta, Jal. Mexico. pp. 45-57.

Guerrero FD, Lovis L, Martins JR (2012). Acaricide Resistance Mechanisms in *Rhipicephalus microplus*. Rev. Bras. Parasitol. 21:1-6.

Jongejan F, Uilenberg G (2004). The global importance of ticks. Parasitology 129:3-14.

Jonsson NN, Hope M (2007). Progress in the epidemiology and diagnosis of amitraz resistance in the cattle tick *Boophilus microplus*. Vet. Parasitol. 146(3):193-198.

Kaljouw M (2008). Resistance to acaricides of Boophilus ticks from cattle in Ghana. Utrecht University, Netherlands.

Kearney (2011). Acaricide resistance in cattle ticks. In: Northern Territory Government. pp. 1-4.

Kemp DH, Gale KR, Nari A, Sabatini GA (1998). Acaricide resistance in the cattle-ticks *Boophilus microplus* and *B. decoloratus*: Review of resistance data; standardization of resistance tests and recommendations for the integrated parasite control to delay resistance. CSIRO Tropical Agriculture, Long Pocket Laboratories, Australia.

Kumar R, Nagar G, Sharma AK, Kumar S, Ray DD, Chaudhuri P, Ghosh S (2013). Survey of pyrethroids resistance in Indian isolates of *Rhipicephalus* (*Boophilus*) microplus: Identification of C190A mutation in the domain II of the para-sodium channel gene. Acta Trop. 125(2):237-245.

Laranja RJ, Martins JR, Cerésér VH, Corrêa BL, Feraz C (1989). Identificação de uma estirpe de *Boophilus microplus* resistente a carrapaticidas piretroides, no Estado de Rio Grande de Sul. Anais VI Semin. Bras. Parasit. Vet. 83.

Leite RC (1988). *Boophilus microplus* (Canestrini, 1887) susceptibilidade, usa atual e retrospectivo de carrapaticidas em propiedades das regiões fisiográficas da Baixada do Grande Rio e Rio de Janeiro. Uma abordagem epidemiológica. In. Universida de Federal Rural do Rio de Janeiro. pp. 1-151.

Li A, Davey R, Miller R, George J (2004). Detection and characterization of amitraz resistance in the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae). J. Med. Entomol. 41:193-200.

Lovis L, Perret J, Bouvier J, Fellay J, Kaminsky R, Betschart B, Sager H (2011). A new *in vitro* test to evaluate the resistance level against acaricides of the cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*. Vet. Parasitol. 182:269-280.

Lovis L, Reggi J, Berggoetz M, Betschart B, Sager H (2013). Determination of Acaricide Resistance in *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) Field Populations of Argentina South Africa and Australia with the Larval Tarsal Test. J. Med. Entomol. 50:326-335.

Madder M, Adehan S, De Deken R, Adehan R, Lokossou R (2012). New foci of *Rhipicephalus microplus* in West Africa 30. Exp. Appl. Acarol. 56:385-390.

Martins RJ, Correa LV, Cereser HV, Arteche PCC (1995). A situation report on resistance to acaricides by the cattle tick Boophilus microplus in the state of Rio Grande Do Sul Southern Brazil. In: III Seminario internacional de parasitologia animal. Resistencia y control en garrapatas y moscas de importancia veterinaria, Acapulco, Gro. Mexico.

Mattioli RC, Bah M, Faye J, Kora S, Cassama M (1993). A comparison of field tick infestation on N'Dama, Zebu and N'Dama × Zebu crossbreed cattle. Vet. Parasitol. 47:139-148.

Mendes EC, Mendes MC, Sato ME (2013). Diagnosis of amitraz resistance in Brazilian populations of *Rhipicephalus* (Boophilus) microplus (Acari: Ixodidae) with larval immersion test. Exp. Appl. Acarol. 61(3):357-369.

Miller RJ, Davey RB, George JE (2002). Modification of the food and agriculture organization larval packet test to measure amitraz-susceptibility against ixodidae. J. Med. Entomol. 39:645-651.

Miller RJ, Martins JR, Ducornez S, Barre N, Solari DMA, Cuore Passegi UD, George JE (2003). Use of a modified-Larval Packet Test (LPT) to measure amitraz susceptibility in Boophilus microplus in Brazil, New Caledonia, and Uruguay, and a comparison of the modified-LPT to a modified-Shaw technique for amitraz testing in *B. microplus*. In: V International Seminar in Animal Parasitology. World situation of parasite resistance in veterinary medicine, SENASICA-INIFAP-INFARVET-UADY-FAO-AMPAVE. Merida, Yucatan,Mexico. pp. 118-123.

Musonge EN, Tanya VN (1987). Testing for tick resistance to various acaricides at Wakwa, Cameroon. Rev. Sci. Technol. Anim. Sci. Ser. 3:129-134

Nolan J (1981). Current developments in resistance to amidine and pyrethroid tickicides in Australia. In: Whitehead GB, Gibson JD (Eds.). Proceedings of Tick Biology and Control Conference. Tick Research Unit, Rhodes University, Grahamston, South Africa. pp. 109-114.

Ritz C, Streibig JC (2005). Bioassay analysis using R. J. Stat. Softw. 12(5):1-22.

Robertson JL, Preisler HK (1992). Pesticide Bioassays with Arthropods CRC Press. Boca Raton 127 p.

Rodriguez-Vivas RI, Alonso-Diaz MA, Rodriguez-Arevalo F, FragosoSa´nchez H, Santamaria, VM, Rosario-Cruz M (2006). Prevalence and potential risk factors for organophosphate and pyrethroid resistance in Boophilus microplus ticks in cattle ranches from the state of Yucatan, Mexico. Vet. Parasitol. 136:335-342.

Rodriguez-Vivas RI, Rivas AL, Chowell G, Fragoso SH, Rosario CR, Garcia Z, Smith SD, Williams JJ, Schwager SJ (2007). Spatial distribution of acaricide profiles (*Boophilus microplus* strains susceptible or resistant to acaricides) in southeastern Mexico. Vet. Parasitol. 146:158-169.

Sanchez-Cespedes M, Parrella P, Esteller M, Nomoto S, Trink B, Engles JM, Westra WH, Herman JG, Sidransky D (2002). Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung. Cancer Res. 62(13):3659-3662.

Soberanes NC, Santamaria MV, Fragoso HS, Garcia ZV (2002). First case reported of amitraz resistance in the cattle tick Boophilus microplus in Mexico. Técnica Pecuaria en México 40(1):81-92.

Taylor RJ, Oberem P (1995). Some characteristics of an Amitraz resistant strain of B. microplus originating from South Africa. In: Coons L, Rotchild M (Eds.). Proceedings of the Second International Conference on Tick-borne Pathogens at the Host-Vector Interface, ruger National Park, South Africa. pp. 1-62.

Temeyer KB, Pound JM, Miller JA, Chen AC, Pruett JH, Guerrero FD, Davey FD, Untalan PM, Lohmeyer KH, Andrew Yl, Miller RJ, George JE (2004). Organophosphate resistance in mexican strains of *Boophilus microplus*: A major threat to the U.S cattle industry. SAAS Bull. Biochem. Biotechnol. 17:43-51.