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Original article

Variation of diazinon and amitraz susceptibility of *Hyalomma marginatum* (Acari: Ixodidae) in the Rabat-Sale-Kenitra region of Morocco

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ABSTRACT

In the present study, the acaricide susceptibility status of *Hyalomma marginatum* in Morocco was investigated in the Rabat-Sale-Kenitra region using the Larval Packet Test. The overall LC_{50} value for diazinon and amitraz was 115 ppm (95% CI: [104; 125]) and 22 ppm (95% CI: [21; 23]), respectively. The LC_{50} values varied significantly between the nine sampled locations ($P<0.001$) ranging from 75 ppm (95% CI: [65; 84]) in Ouelmes to 179 ppm (95% CI: [139; 201]) in Jorf El Melha for diazinon and from 18 ppm (95% CI: [15; 21]) in Skhirat to 28 ppm (95% CI: [24; 31]) in Ouelmes for amitraz. Sequencing of the target-site of diazinon, acetylcholinesterase 1 (AChE1), indicated that previously reported resistance mutations in AChE1 were absent in ticks from Jorf El Melha surviving 500 ppm diazinon. This study is the first report on the *H. marginatum* susceptibilty status to the most frequently used acaricides in Morocco and indicates that acaricide tick resistance is emerging.

1. Introduction

Hyalomma ticks are widely distributed in warm to hot and dry biotopes (Santos-Silva and Vatansever, 2017). In Morocco, *Hyalomma marginatum* (or the Mediterranean *Hyalomma*) is the most prevalent tick species found on cattle in various bio-climatic regions throughout the country (Walker et al., 2003). It is one of the main vectors of bovine tropical theileriosis and piroplasmosis in cattle (Jongejan et al., 1983; Apanaskevich and Horak, 2008). Moreover, *H. marginatum* is the main vector of the Crimean-Congo hemorrhagic fever virus to humans and is also responsible for its emergence in previously uninfected regions including Morocco (Zeller et al., 1994; Palomar et al., 2013; Chitimia--Dobler et al., 2019). The commonly used method to control ticks is the application of chemicals. Diazinon and amitraz are frequently used acaricides to prevent tick infestation of cattle in Morocco. Diazinon is an organophosphate which acts as an irreversible inhibitor of acetylcholinesterase leading to the death of the arthropod (Aldridge, 1950). Amitraz is a formamidine and is characterized by its rapid knock-down effect on ticks. The active metabolite of amitraz, N(2)-(2, 4-Dimethylphenyl)-N(1)-methyformamidine (DPMF), exerts its acaricidal effects through its agonistic action on octopamine receptors. Upon binding to these receptors, cAMP levels rise, triggering excitatory effects, including convulson and tremors, which ultimately leads to the death of the tick (Davenport et al., 1985; Baxter and Barker, 1999; Kita et al., 2017; Takata et al., 2020). For both compounds, target-site resistance mutations have been reported in resistant tick populations (Chen et al., 2007; Temeyer, 2018). Tick resistance to diazinon and amitraz has been documented in many regions of the world (Miller et al., 2002; Li et al., 2004; Chevillon et al., 2007; Kumar et al., 2011a; Mendes et al., 2013; Petermann et al., 2016; Dutta et al., 2017; Klafke et al., 2017; Kumar et al., 2020; Sagar et al., 2020). However, in Morocco there is virtually no information on the tick susceptibilty status against

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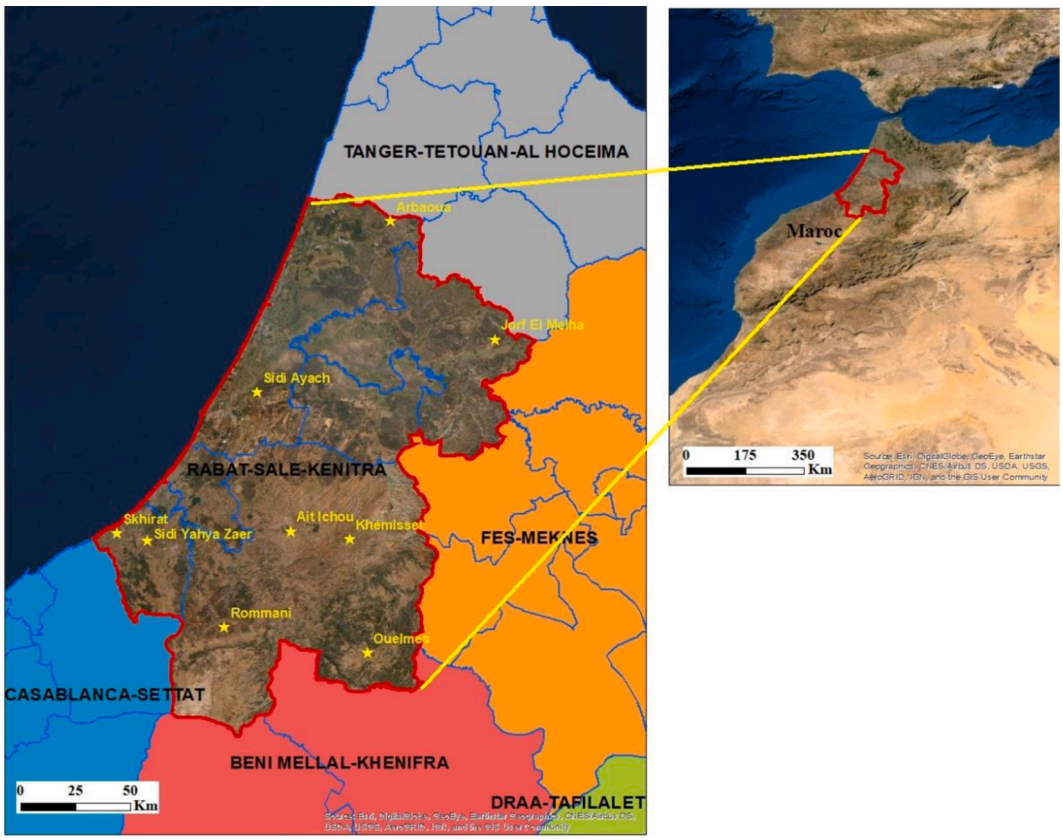

Fig. 1. The nine locations of the study in the Rabat-Sale-Kenitra region-Morocco.

diazinon and amitraz. The aim of this study is to evaluate the efficacy of diazinon and amitraz in *H. marginatum* ticks collected from different agro-climatic locations in the Rabat-Sale-Kenitra region of Morocco.

2. Materials and methods

2.1. Study sites

The present study was conducted in the Rabat-Sale-Kenitra region located in the northwest of Morocco. The climate of the region is of semi-arid Mediterranean type with maritime or continental oceanic influence: mild, moderate and rainy in winter and humid and temperate in summer with hot and dry days (Csa according to the Köppen-Geiger classification). The average annual minimum temperature is 4 °C and the average annual maximum is 40 °C, and the annual rainfall varies between 300 mm and 900 mm. In addition to agriculture, livestock has an important role, especially intensive cattle farming, with almost 520,000 heads of cattle. Samples were collected from 9 locations well distributed in different agro-ecological zones in the Rabat-Sale-Kenitra region (Fig. 1). Skhirat (SKH), Sidi Yahya Zaer (SYZ) and Rommani (ROM) belong to the Coastal plains, where agriculture is based on rains and characterized by a cereal culture associated with increasingly intensive cattle production (milk and beef) and a mixture between subsistence farms and large farms. Jorf El Melha (JEM), Sidi Ayach (SAY) and Arabaoua (ARB) are located in the Rharb plains characterized by a well-developed irrigation system with consequently diversified agriculture (crops, cereals, oleaginous plants and legumes) and a high concentration of dairy cattle and large farms. Khemisset (KHM) is located in north western Morocco, 80 km from the capital between zaer plains and the Middle Atlas. Khemisset is well known for its highly developed agriculture and cattle farming. Ouelmes (OLM) and Ait Ichou (ACH) are part of the Middle Atlas, which is a mountainous region where crops are integrated with livestock and which is further characterized by traditional farming of cattle and summer transhumance of sheep and goat flocks.

2.2. Tick collection

At each location, ten cattle farms (except for OLM with nine farms) with tick infestation and whose owners agreed to collaborate, were selected for tick collection. From each farm, one engorged *H. marginatum* tick isolate with good quality (alive, high ability to move, having an integral body and weighing between 150 and 350 mg) (FAO, 2004) was selected to ensure that enough offspring, i.e., larvae, would be available to run the Larval Packet Test in triplicate for each acaricide. Therefore, 10 farms per location (9 for OLM) were independently assessed for resistance, and thus correspond to the replications for each location.

Samples were collected between April and August 2019. Engorged female ticks were collected separately from restrained cows using forceps and were put in individual containers with a few small holes allowing air to circulate. Upon arrival at the laboratory, ticks were washed in distilled water, identified based on morphological characteristics (Estrada-Peña et al., 2004; Apanaskevich and Horak, 2008), weighed and kept in filter paper in individual tubes, covered with a muslin cloth and secured with a perforated lid for egg-laying.

The study protocol was approved by the Ethical Committee for Biomedical Research of the Mohammed V University of Rabat, Morocco (n°627; July 2019).

2.3. Tick maintenance

Engorged female ticks were maintained in desiccators under 84% relative humidity (RH) created by a saturated salt solution (Winston and Bates, 1960). Desiccators were held in an incubator to maintain ticks at 28 °C for oviposition. The freshly laid eggs were aliquoted in separate tubes (50 mg/tube amounting to approximately 1000 larvae/tube) and kept at the same conditions of temperature and relative humidity until

hatching. Hatched larvae were used for bioassays.

2.4. Assessment of chemical acaricides efficacy by the Larval Packet Test

Susceptibility to diazinon and amitraz (≥ 98.0% purity, Sigma-Aldrich, Belgium) was assessed using the Larval Packet Test (LPT). The LPT was first described by Stone and Haydock (1962) and was recommended by the Food and Agriculture Organization (FAO, 1971) as a standard bioassay for testing tick resistance to organophosphates and formamidines. Briefly, a 10,000 ppm stock solution was prepared by dissolving an appropriate amount of technical grade acaricide into a solution of two parts trichloroethylene (TCE) and one part olive oil (OO) (Sigma-Aldrich, Germany). Next, the stock solution was serially diluted (using 2 parts TCE/1 part OO) to obtain seven acaricide concentrations: 5000, 1000, 500, 200, 100, 40, and 8 ppm. Each acaricide concentration and a control (2 parts TCE/1 part OO) was tested on 15–20-day-old larvae and three replicates were performed for each concentration. Using a micropipette, a volume of 0.67 ml of each dilution was applied to a Whatman filter paper (Cat No. 3001 917) of size 8.5 × 7.5 cm, starting by the lowest and ending with the highest concentration. Filter papers were then dried under a fume hood in a constant airflow for 2 h to evaporate the TCE. After drying, each filter paper was folded in half. Filter paper sides were then clipped using 2 bulldog clips, and approximately 100 larvae were placed in each packet using a paintbrush. The open end of the packet was secured with a third bulldog clip. Once assembled, the packets were suspended on a metal rod and held in a humidified incubator at 25 ± 1 °C, 84% RH. Following a 24-hour incubation period for diazinon and a 48-hour incubation period for amitraz (FAO, 2004), packets were removed and mortality was assessed by counting the number of alive and dead ticks in each packet. Dead larvae were characterized by a lack of movement after being disturbed and appearing flat and desiccated. Moribund larvae that demonstrated a lack of movement were categorized as dead. Larvae that were dead because they were crushed by the bulldog clips were not included in the overall count

2.5. Statistical analysis

The dose-response curve was modeled by the common 4-parameter log logistic model with density function:

$$f(x) = c + \frac{d - c}{1 + (x/e)^b} \quad (1)$$

where x was the dose, c and d were the lower and upper limits, e was the LC_{50} and b reflects the steepness of the curve around LC_{50}. In our models, we fitted the 3-parameter log-logistic model using the R-package DRC version 3.0–1 with the upper limit, i.e., d, put equal to 100%. For each tick isolate from a farm within a location, a separate dose-response curve was fitted and the LC_{50} was determined. The analysis for the variation in resistance as a function of location was based on a meta-analytic approach. We first derived the LC_{50} values and their variance for each different tick isolate within a location and used these as an input for the meta-analysis. We used a likelihood ratio test to assess whether the variation between locations was significant. Results of the meta-analysis were presented in forest plots. Finally, the LC_{50} values of the different locations were compared pairwise and testing was based on the 95% confidence interval.

To the best of our knowledge, there is no susceptible reference tick line of *H. marginatum* for resistance characterization available. For this reason we used the LC_{50} value of the location which harbored the most susceptible ticks as a reference to calculate the resistance ratios (RR) of the different locations.

Table 1
Primers used for PCR amplification of AChE1 and sequencing.

Primer name	Sequence (5′−3′)	Tm (°C)	Amplicon size (bp)
AChE1_F	CCGGTGGAAACCGTGGTAGTAGA	63.8	1426
AChE1_R	TAGCCCAGTACCGCATGAGTCG	64.09	
AChE1_S1	GTCGCCTCTCTCGGGTTCCT		
AChE1_S2	TCCGAAGCAGGCGATGTTCT		
AChE1_S3	GAAGGTTCTTGGTTCGTGCAGT		

Table 2
Resistance ratios and its respective 95% CI for Diazinon and Amitraz at the different locations with the location with the lowest LC50 value as reference.

Location	Resistance ratio Diazinon	Amitraz
ACH	1	1.401 [1.163; 1.640]
ARB	2.135 [1.552; 3.417]	1.334 [1.108; 1.560]
JEM	2.339 [1.708; 3.711]	1.086 [0.888; 1.284]
KHM	1.662 [1.191; 2.748]	1.067 [0.871; 1.262]
OLM	1.041 [0.704; 1.996]	1.500 [1.252; 1.748]
ROM	1.091 [0.749; 2.013]	1.146 [0.944; 1.349]
SAY	1.695 [1.216; 2.798]	1.244 [1.027; 1.460]
SKH	1.627 [1.162; 2.712]	1
SYZ	1.633 [1.168; 2.710]	1.111 [0.941; 1.354]

2.6. DNA extraction, pcr amplification and sequencing of the AChE1 gene

In the LPT dose-response experiment of one of the farms of JEM, those larvae that were either alive at 500 ppm diazinon or were dead at 8 ppm diazinon were collected and preserved in 70% ethanol (sample JEM3-A500 and JEM3-D8, respectively). Twenty larvae from both samples were allowed to dry on Whatman filter paper and collected in a 1.5-mL Eppendorf tube. Next, 5 µL proteinase K (20 mg/mL) and 100 µL lysis buffer (10 µM Tris–HCl pH 8,3, 50 µM KCl, 0.5% Tween 20) was added, larvae were crushed using a pestle and genomic DNA was isolated by standard phenol/chloroform DNA extraction (Green and Sambrook, 2014) and dissolved in 20 µL distilled water.

The AChE1 gene sequence of *Hyalomma dromedarii* was identified by performing a tBLASTn search against the *H. dromedarii* transcriptome (Bensaoud et al., 2018) and using *Rhipicephalus microplus* AChE1 (GenBank accession CAA11702, Van Zee and Hill, 2017) protein sequence as query. Presuming that the AChE1 gene structure (intron/exon boundaries) between *H. dromedarii* and the related *R. microplus* is conserved, primers were designed to amplify 1426 bp of a large exon of *H. dromedarii* AChE1.

Next, assuming that there is a high similarity between the *H. marginatum* and *H. dromedarii* AChE1 coding sequence, these *H. dromedarii* AChE1 primers were used in a PCR to amplify the corresponding AChE1 region in *H. marginatum*. PCRs were performed in a volume of 10 µl per sample, containing 2 µl of DNA template (JEM3-A500 or JEM3-D8), 0.1 µl of TEMPase Hot Start DNA Polymerase (5 U/µl; VWR, Leuven, Belgium), 0.2 µl of dNTP Mix (10 mM each; Bioline, London, UK), 1 µl of AChE1 primers (AChE1_F and AChE1_R, 5 µM each), 1 µl of 10x Key Buffer (VWR), and 5.7 µl of ultrapure water. The PCR program consisted of an initiation step of 14 min 30 s at 95 °C followed by 40 amplification cycles (denaturation for 30 s at 95 °C, annealing for 30 s at 62 °C, and extension for 1 min 30 s at 72 °C) and a final elongation step of 5 min at 72 °C. PCR amplicons were analyzed via agarose gel electrophoresis. Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with primer AChE1_F and three internal sequencing primers (AChE1_SX primers, see Table 1) and run at Eurofins Genomics (Ebersberg, Germany).

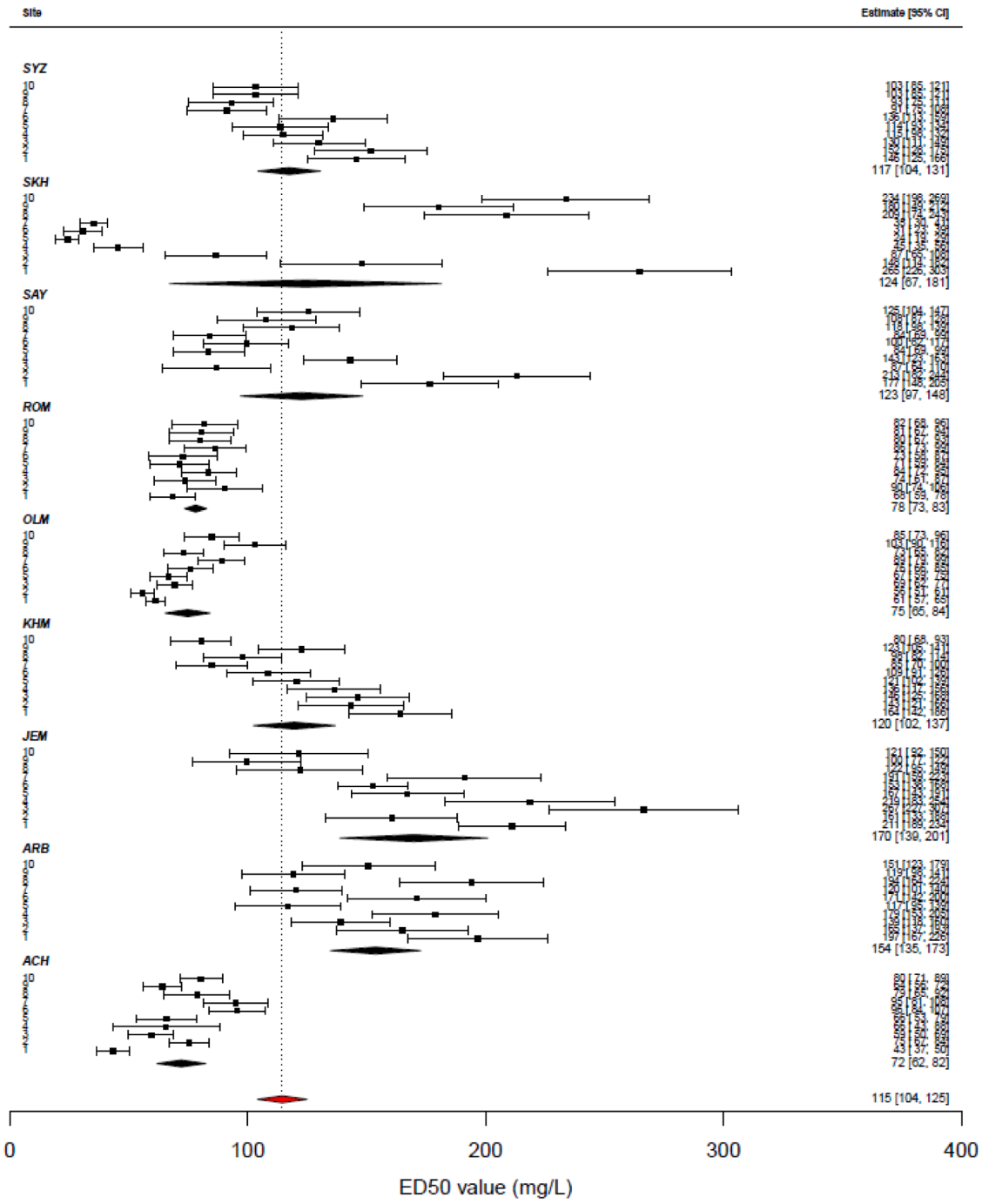

Fig. 2. The forest plot for the ED_{50} values of diazinon for *Hyalomma marginatum*. The rectangles correspond to the ED_{50} values of individual sampling sites, the diamonds for the overall ED_{50} at a location or overall (in red).

3. Results

3.1. Diazinon

The LC_{50} values differ significantly between the nine locations ($P<0.001$). The lowest LC_{50} value is found for ACH and is thus taken as a reference value to derive the RRs. The highest RR is found for JEM and equals 2.34 (95% CI: [1.71; 3.71]) (Table 2). The forest plot is presented in Fig. 2. The overall LC_{50} value equals 115 ppm (95% CI: [104; 125]). The different dose-response curves for the 10 different samples per location are given in Appendix A, Fig. A1, together with the overall dose-response curve for the particular location. Most dose-response curves within a location are similar, except for the SKH region, where much more variation was detected between the dose-response curves, for instance between SKH5 with LC_{50} equal to 24.29 ppm (95% CI: [19.33; 29.24]) and SKH1 with LC_{50} equal to 264.68 ppm (95% CI: [225.92; 303.44]).

3.2. Amitraz

The LC_{50} values differ significantly between the nine locations ($P<0.001$). The lowest LC_{50} value is found for SKH and equals 18 (95% CI: [15; 21]) and is thus taken as reference value to derive the RRs. The highest RR is found for OLM equal to 1.50 (95% CI: [1.25; 1.75]) (Table 2). The overall LC_{50} value equals 22 ppm (95% CI: [21; 23]). The forest plot is presented in Fig. 3. The different dose-response curves for

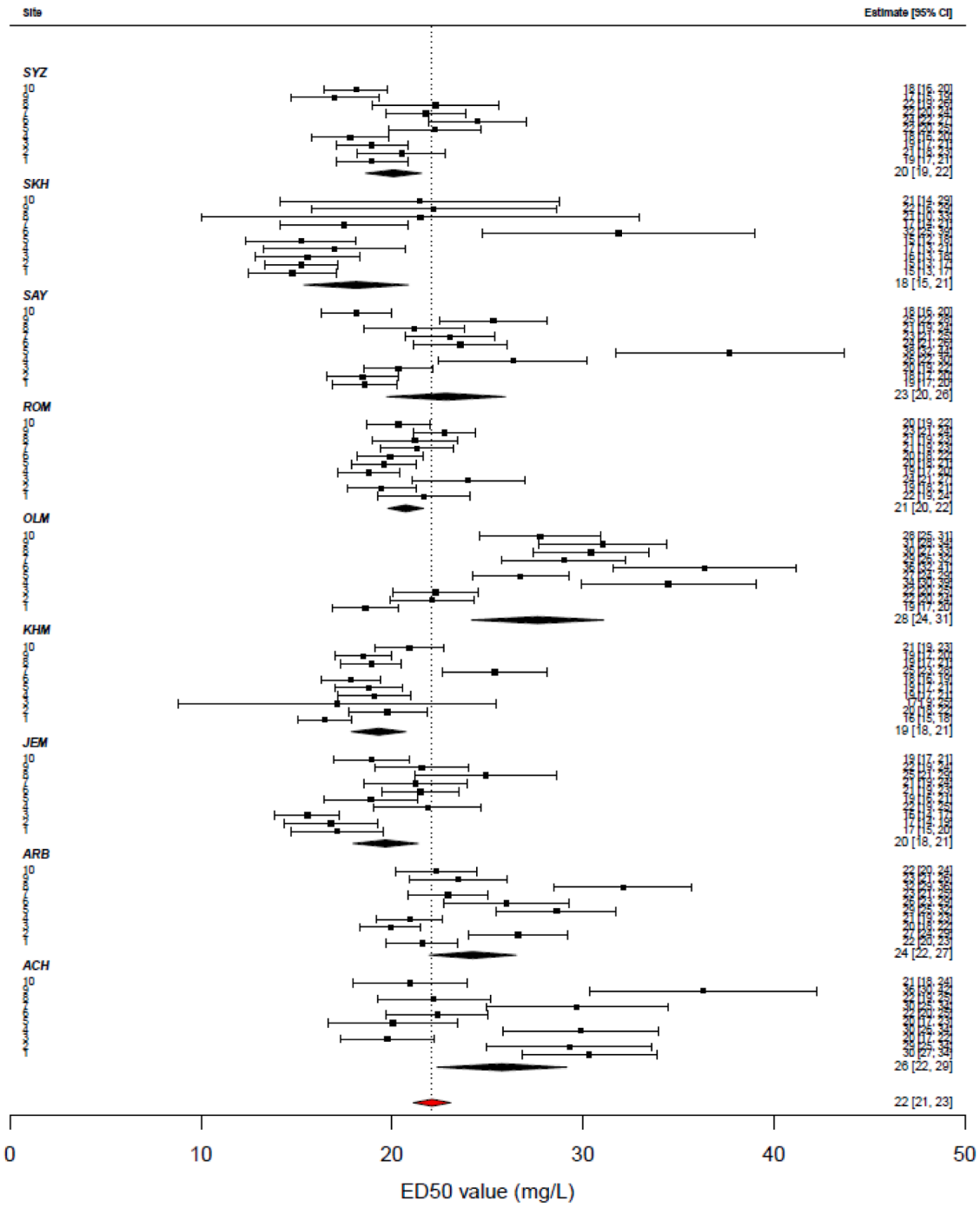

Fig. 3. The forest plot for the ED_{50} values of amitraz for *Hyalomma marginatum*. The rectangles correspond to the ED_{50} values of individual sampling sites, the diamonds for the overall ED_{50} at a location or overall (in red).

the 10 different samples per location are given in Appendix A, Fig. A2, together with the overall dose-response curve for the particular location. All dose-response curves within a location are similar.

3.3. Sequencing of the AChE1 gene

PCR successfully amplified a large fragment (>1400 bp) of the AChE1 coding sequence of *H. marginatum*. This fragment was amplified and sequenced for both JEM3-D8 (dead at 8 ppm diazinon) and JEM3-A500 (alive at 500 ppm diazinon), and submitted to the NCBI database (GenBank accession: MT743247 (JEM3-D8) and MT743248 (JEM3-A500). A single nucleotide polymorphism, T138G (*H. marginatum* partial AChE1 numbering), was identified in JEM3-A500 compared to JEM3-D8, resulting in a substitution (ATT (isoleucine) ->AGT (serine) at a residue that aligns between residue 49 (glutamate) and 50 (glycine) of the mature AChE protein of Torpedo/Tetronarce californica (Fig. 4). Inspecting the sequencing chromatograms, revealed that both 138T and 138 G were present in JEM3-D8, while 138 G was fixed in JEM3-A500. Mining the ESTHER database (Lenfant et al., 2012) revealed that AChE resistance mutations at T. californica AChE residue positions lower than 57 have not been reported yet (see Biological Data -> Mutations -> Queries for Insecticide Resistance at the ESTHER database).

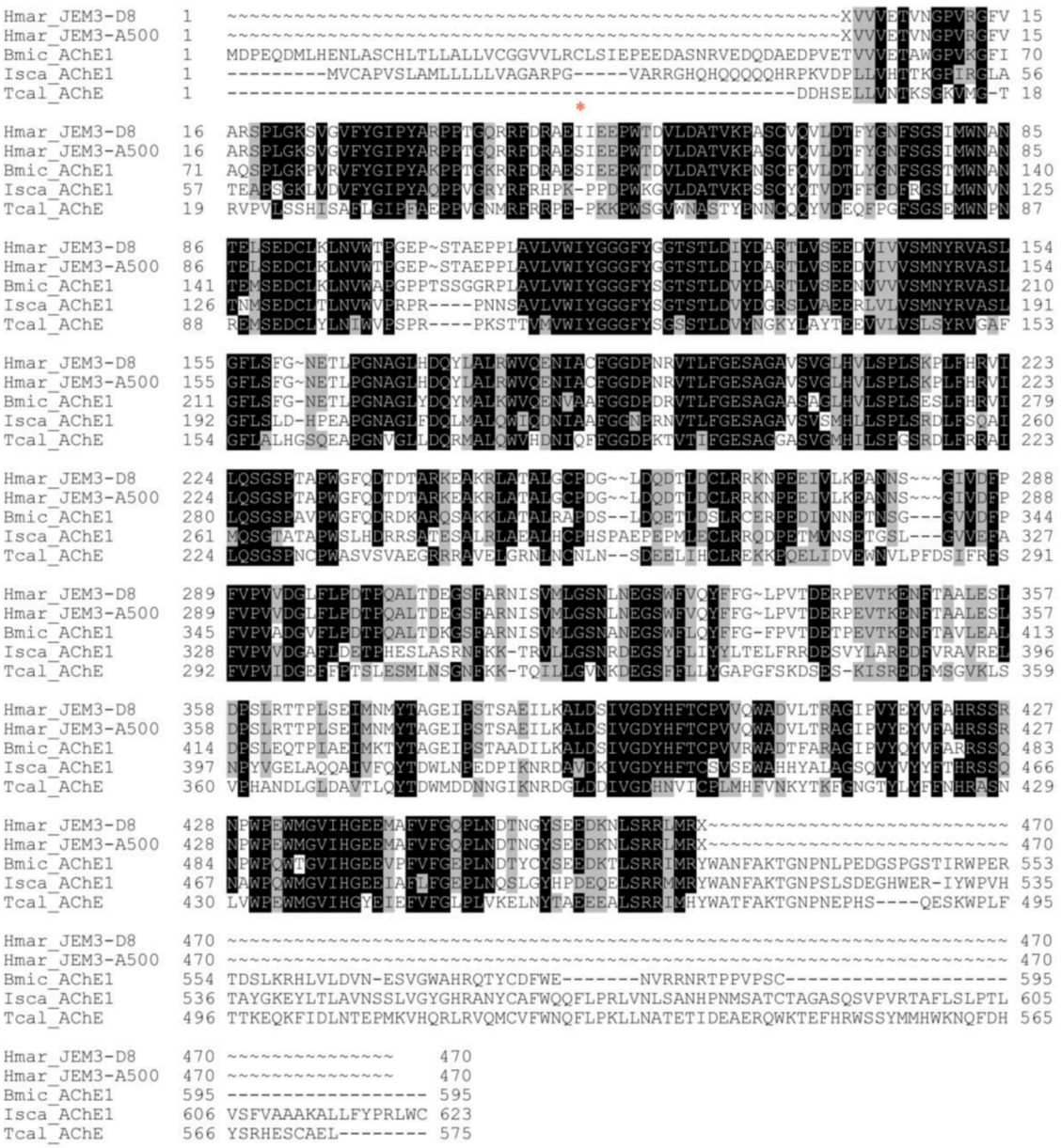

Fig. 4. Clustal-W alignment of the partial AChE1 protein sequence of *Hyalomma marginatum* JEM3-D8 (larvae dead at 8 mg a.i. diazinon/L) and JEM3-A500 (alive at 500 mg a.i. diazinon/L) with AChE1of *Rhipicephalus microplus* (Rmic,GenBank accession: CAA11702) and *Ixodes scapularis* (Isca, NCBI Reference Sequence XP_029832476.1) and AChEof *Torpedo/Tetronarce californica* (Tcal, GenBank accession: CAA27169.1). The I49/50S substitution in JEM3-A500 compared to JEM3-D20 is indicated with a red asterisk.

4. Discussion

In Morocco, ticks and tick-transmitted diseases present a huge constraint to the development of the livestock sector. In addition to the decreased production and the high mortality in cows, acaricide treatment is expensive for the farmers. Besides, the use of acaricides is without proper regulation or control strategy and no study has been performed before to evaluate the efficacy of the used acaricides.

For both diazinon and amitraz, the LC_{50} values differ significantly between the locations, and therefore susceptiblity varies in this region, which is an indicator that resistance is either present or ermerging. The range of RRs for diazinon (1.09 to 2.34) is much larger than that for amitraz (1.07 to 1.50). Similar results have been reported for amitraz by Jyoti et al. (2019) for *Hyalomma anatolicum* (RRs ranged from 0.83 to 1.52). In the same study four tick isolates showed moderate levels of resistance and one tick isolate was susceptible to this compound.

JEM3 tick isolate had an LC_{50} of more than 250 ppm diazinon (Fig. 2) and was therefore selected to screen for the presence of mutations in the AChE gene, encoding the target-site of diazinon. In *R. microplus*, three AChE genes have been identified, with one gene being orthologous to AChE1 of insects (Temeyer et al., 2010; Bendele et al., 2015; Van Zee and Hill, 2017). Given the well-documented role of AChE1 mutations in organophosphate resistance in insects and mites (Van Leeuwen et al., 2010; Feyereisen et al., 2015), we only focused on the AChE1 gene of *H. marginatum* and PCR-amplified a large AChE1 coding fragment of JEM3 larvae that, based on the LPT dose-response experiments, either survived a dose of 500 ppm (JEM3-A500) or died at a dose of 8 ppm (JEM3-D8). A single nucleotide polymorphism was detected between sequences of both samples, but the resulting amino acid change (I49/50S) was located at a residue that is not conserved across ticks and other arthropod species (Bendele et al., 2015) and has not yet been reported to be associated with organophosphate resistance (Lenfant et al.,

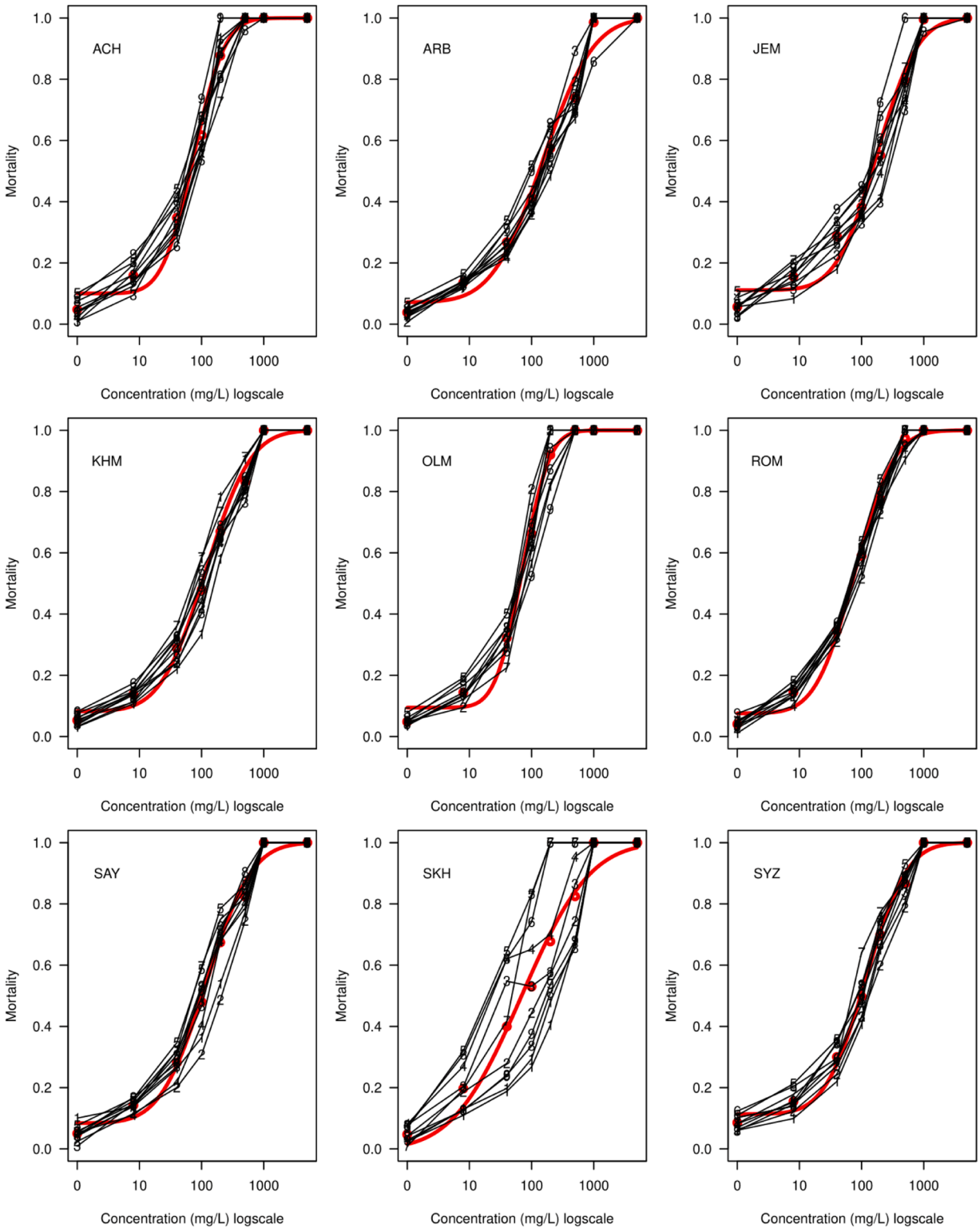

Fig. A1. Diazinon dose-response curves for the 10 different sampling sites per location with the overall dose-response curve for each location in red.

2012), and was also not among previously identified AChE1 mutations in *R. microplus* (Temeyer et al., 2010; Bendele et al., 2015). Besides, JEM3-A500 larvae had a serine at position 49/50, and almost all *R. microplus* AChE1 sequences in the NCBI database also have a serine at that position (data not shown), suggesting it is unlikely that this nucleotide polymorphism is associated with resistance, and possibly other resistance mechanisms (e.g. mutations in other AChE genes (AChE2 or AChE3) or metabolic resistance mechanisms) are at play.

Hyalomma marginatum is a two-host tick, with larvae and nymphs feeding on small mammals and ground-dwelling birds and adults feeding on large mammals (Santos-Silva and Vatansever, 2017). In comparison with one-host ticks such as *R. microplus* (Kumar et al., 2011b; Muyobela et al., 2015; Sagar et al., 2020; Villar et al., 2019), *H. marginatum* showed low resistance levels. Previous studies have also shown low resistance levels in multi-host ticks compared to one-host ticks (Shyma et al., 2012, 2013; Singh et al., 2014; Gaur et al., 2016,

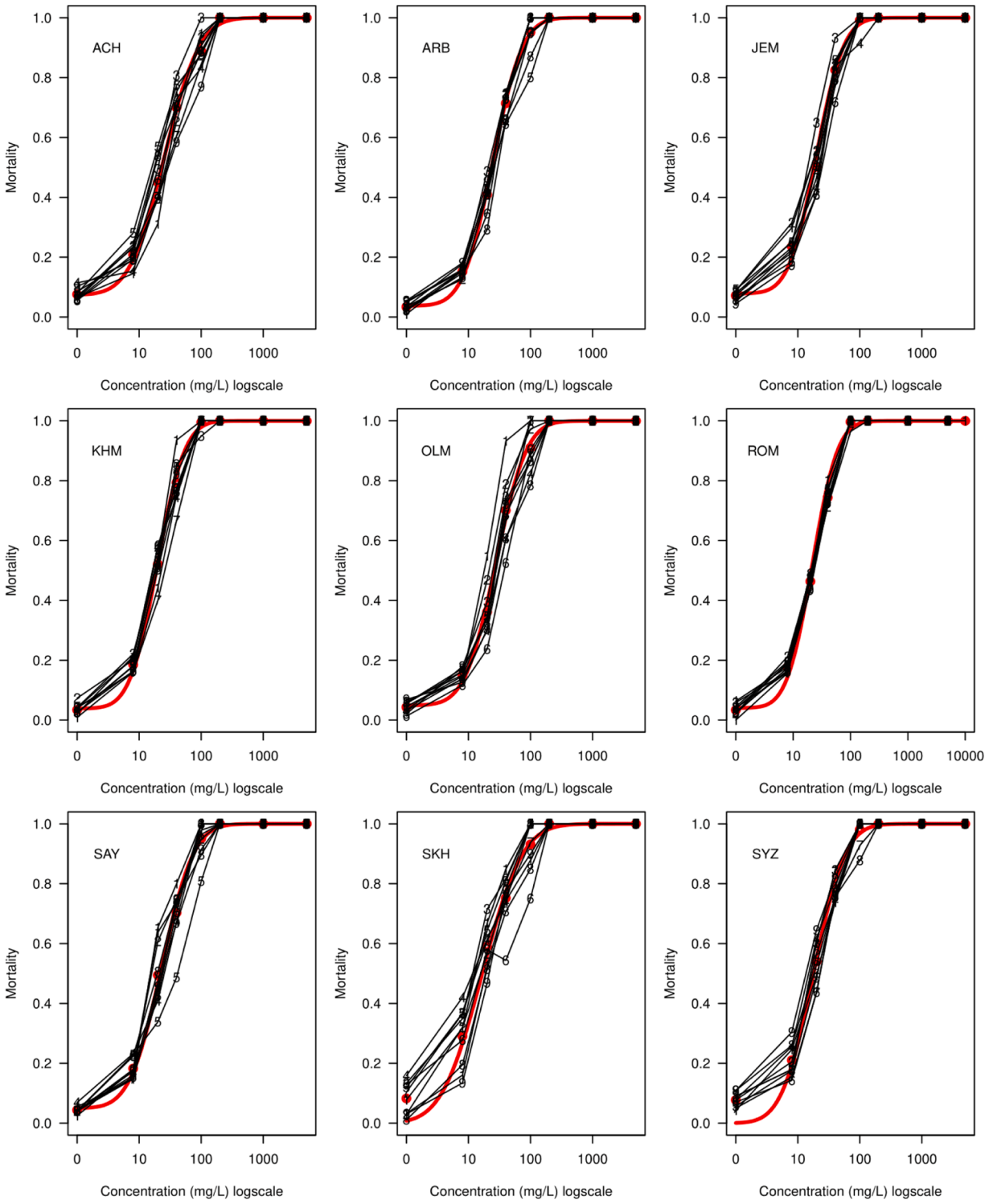

Fig. A2. Amitraz dose-response curves for the 10 different sampling sites per location with the overall dose-response curve for each location in red.

Singh et al., 2019; Jyoti et al., 2019). According to Shyma et al. (2012) this is due to the short life cycle duration and the exposure of all stages of a one-host tick to the acaricide treatment. In the case of *H. marginatum*, immature stages are not exposed to the chemical as they do not feed on cows. In addition, *H. marginatum* has a long life cycle and produces only one generation per year. This could explain the low occurrence of resistance in this tick species. However, based on the observed differences between ticks from different locations, there is a risk of further development of resistance in *H. marginatum* in this region in Morocco.

5. Conclusion

This study reports on differences in the susceptibility of *H. marginatum* towards diazinon and amitraz, commonly used acaricides in Morocco, and suggests that *H. marginatum* acaricide resistance is emerging. Hence, suitable strategies to avoid further tick control failure in Morocco should be considered. Lastly, tick resistance monitoring should be extended to other parts of Morocco.

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Credit author statement

Latifa Elhachimi: design and conceptualization, performing experiments, interpreting data and writing the draft and final version of the paper.

Luc Duchateau: design and conceptualization, supervising, analyzing and interpreting the data.

Sahibi Hamid: design and conceptualization, supervising

Thomas Van Leeuwen and **Wannes Dermauw**: supporting the design conceptualization and execution of experimental protocols, supervising the molecular biology work.

Carolien Rogiers: supporting execution of laboratory work.

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Appendix A

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2021.101883.

References

Aldridge, W.N., 1950. Some properties of specific cholinesterase with particular reference to the mechanism of inhibition by diethyl p-nitrophenyl thiophosphate (E 605) and analogues. Biochem. J. 46, 451.

Apanaskevich, D.A., Horak, I.G., 2008. The genus *Hyalomma* Koch, 1844: V. Re-evaluation of the taxonomic rank of taxa comprising the *H.* (*Euhyalomma*) *marginatum* Koch complex of species (Acari: ixodidae) with redescription of all parasitic stages and notes on biology. Int. J. Acarol. 34, 13–42.

Baxter, G.D., Barker, S.C., 1999. Isolation of a cDNA for an octopamine-like, G-protein coupled receptor from the cattle tick, *Boophilus microplus*. Insect Biochem. Mol. Biol. 29, 461–467.

Bendele, K.G., Guerrero, F.D., Miller, R.J., Li, A.Y., Barrero, R.A., Moolhuijzen, P.M., Black, M., MacCooke, J.K., Meyer, J., Hill, C.A., Bellgard, M.I., 2015. Acetylcholinesterase 1 in populations of organophosphate-resistant North American strains of the cattle tick, *Rhipicephalus microplus* (Acari: ixodidae). Parasitol. Res. 114, 3027–3040.

Bensaoud, C., Nishiyama, M.Y., Hamda, C.B., Lichtenstein, F., de Oliveira, U.C., Faria, F., Junqueira-de-Azevedo, I.L.M., Ghedira, K., Bouattour, A., M'Ghirbi, Y., Chudzinski-Tavassi, A.M., 2018. De novo assembly and annotation of *Hyalomma dromedarii* tick (Acari: ixodidae) sialotranscriptome with regard to gender differences in gene expression. Parasit. Vectors. 11, 1–16.

Chen, A.C., He, H., Davey, R.B., 2007. Mutations in a putative octopamine receptor gene in amitraz- resistant cattle ticks. Vet. Parasitol. 148, 379–383.

Chevillon, C., Ducornez, S., De Meeûs, T., Koffi, B.B., Gaïa, H., Delathière, J.M., Barré, N., 2007. Accumulation of acaricide resistance mechanisms in *Rhipicephalus* (*Boophilus*) *microplus* (Acari: ixodidae) populations from New Caledonia Island. Vet. Parasitol. 147, 276–288.

Chitimia-Dobler, L., Schaper, S., Rieß, R., Bitterwolf, K., Frangoulidis, D., Bestehorn, M., Springer, A., Oehme, R., Drehmann, M., Lindau, A., Mackenstedt, U., Strube, C., Dobler, G., 2019. Imported *hyalomma* ticks in Germany in 2018. Parasit. Vectors. 12, 134.

Davenport, A.P., Morton, D.B., Evans, P.D., 1985. The action of formamidines on octopamine receptors in the locust. Pestic. Biochem. Physiol. 24, 45–52.

Dutta, S., Godara, R., Katoch, R., Yadav, A., Katoch, M., Singh, N.K., 2017. Detection of amitraz and malathion resistance in field populations of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: ixodidae) in Jammu region of India. Exp. Appl. Acarol. 71, 291–301.

Estrada-Peña, A., Bouattour, A., Camicas, J.L., Walker, A.R., 2004. Ticks of Domestic Animals in the Mediterranean region. University of Zaragoza, Spain.

FAO, 1971. Recommended methods for the detection and measurement of resistance of agricultural pest to pesticides-tentative method for larvae of cattle ticks, *Boophilus microplus* spp. FAO method no. 7. FAO Plant Prot. Bull. 19, 15–18.

FAO, 2004. Resistance Management and Integrated Parasite Control in Ruminants-Guidelines, Module 1-Ticks: Acaricide Resistance: Diagnosis, Management and Prevention. Food and Agriculture Organization Animal Production and Health Division, Rome, p. 53.

Feyereisen, R., Dermauw, W., Van Leeuwen, T., 2015. Genotype to phenotype, the molecular and physiological dimensions of resistance in arthropods. Pestic. Biochem. Physiol. 121, 61–77.

Gaur, R.S., Sangwan, A.K., Sangwan, N., Kumar, S., 2016. Acaricide resistance in *Rhipicephalus* (*Boophilus*) *microplus* and *Hyalomma anatolicum* collected from Haryana and Rajasthan states of India. Exp. Appl. Acarol. 69, 487–500.

Green, H., Sambrook, J., 2014. Molecular Cloning: A Laboratory Manual, Fourth ed., p. 1890 https://www.cshlpress.com/default.tpl?cart=159423017913091839 28&fromlink=T&linkaction=ull&linksortby=oop_title&-eqSKUdatarq=934.

Singh, Jyoti, Singh, N.K., Rath, H., S, S., 2019. Modified larval packet test based detection of amitraz resistance in Hyalomma anatolicum Koch (Acari: ixodidae) from Punjab districts of India. Int. J. Acarol. 45, 391–394.

Jongejan, F., Morzaria, S.P., Mustafa, O.E.H., Latif, A.A., 1983. Infection rates of *Theileria annulata* in the salivary glands of the tick *Hyalomma marginatum rufipes*. Vet. Parasitol. 13, 121–126.

Kita, T., Hayashi, T., Ohtani, T., Takao, H., Takasu, H., Liu, G., Ohta, H., Ozoe, F., Ozoe, Y., 2017. Amitraz and its metabolite differentially activate α-and β-adrenergic-like octopamine receptors. Pest Manag. Sci. 73, 984–990.

Klafke, G., Webster, A., Agnol, B.D., Pradel, E., Silva, J., de La Canal, L.H., Becker, M., Osorio, F.M., Mansson, M., Barreto, R., Scheffer, R., 2017. Multiple resistance to acaricides in field populations of *Rhipicephalus microplus* from Rio Grande do Sul state, Southern Brazil. Ticks Tick. Dis. 8, 73–80.

Kumar, R., Paul, S., Kumar, S., Kumar Sharma, A., Gupta, S., Singh Rawat, A.K., Chaudhuri, P., Ray, D.D., Ghosh, S., 2011b. Detection of specific nucleotide changes in the hypervariable region of 16S rDNA gene of *Rhipicephalus (Boophilus)* microplus and *Hyalomma anatolicum* anatolicum. Indian J. Anim. Sci. 81, 1204.

Kumar, S., Paul, S., Sharma, A.K., Kumar, R., Tewari, S.S., Chaudhuri, P., Ray, D.D., Rawat, A.K.S., Ghosh, S., 2011a. Diazinon resistant status in *Rhipicephalus (Boophilus)* microplus collected from different agro climatic regions of India. Vet. Parasitol. 181, 274–281.

Kumar, R., Sharma, A.K., Ghosh, S., 2020. Menace of acaricide resistance in cattle tick, *Rhipicephalus microplus* in India: status and possible mitigation strategies. Vet. Parasitol. 278, 108993.

Lenfant, N., Hotelier, T., Velluet, E., Bourne, Y., Marchot, P., Chatonnet, A., 2012. ESTHER, the database of the α/β-hydrolase fold superfamily of proteins: tools to explore diversity of functions. Nucleic Acids Res 41 (D1), D423–D429.

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

Mendes, E.C., Mendes, M.C., Sato, M.E., 2013. Diagnosis of amitraz resistance in Brazilian populations of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: ixodidae) with larval immersion test. Exp. Appl. Acarol. 61, 357–369.

Miller, R.J., Davey, R.B., George, J.E., 2002. Modification of the food and agriculture organization larval packet test to measure amitraz-susceptibility against Ixodidae. J. Med. Entomol. 39, 645–651.

Muyobela, J., Nkunika, P.O.Y., Mwase, E.T., 2015. Resistance status of ticks (Acari; Ixodidae) to amitraz and cypermethrin acaricides in Isoka District, Zambia. Trop. Anim. Health Prod. 47, 1599–1605.

Palomar, A.M., Portillo, A., Santibáñez, P., Mazuelas, D., Arizaga, J., Crespo, A., Gutiérrez, O., Cuadrado, J.F., Oteo, J.A., 2013. Crimean-Congo hemorrhagic fever virus in ticks from migratory birds. Morocco. Emerg. Infect. Dis. 19, 260–263.

Petermann, J., Cauquil, L., Hurlin, J.C., Gaia, H., Hüe, T., 2016. Survey of cattle tick, *Riphicephalus* (*Boophilus*) *microplus*, resistance to amitraz and deltamethrin in New Caledonia. Vet. Parasitol. 217, 64–70.

Sagar, S.V., Saini, K., Sharma, A.K., Kumar, S., Kumar, R., Fular, A., Shakya, M., Upadhaya, D., Nagar, G., Shanmuganath, C., Samanta, S., Kumar, S., Ghosh, S., 2020. Acaricide resistance in *Rhipicephalus microplus* collected from selected districts of Madhya Pradesh, Uttar Pradesh and Punjab states of India. Trop. Anim. Health Prod. 52, 611–618.

Santos-Silva, M.M., Vatansever, Z., 2017. *Hyalomma Marginatum* Koch, 1844 (Figs. 139–141). In Ticks of Europe and North Africa. Springer, Cham, Switzerland, pp. 349–354.

Shyma, K.P., Kumar, S., Sangwan, A.K., Sharma, A.K., Nagar, G., Ray, D.D., Ghosh, S., 2013. Acaricide resistance status of *Rhipicephalus* (*Boophilus*) *microplus* and *Hyalomma anatolicum* collected from Haryana. Indian J. Anim. Sci. 83, 591–594.

Shyma, K.P., Kumar, S., Sharma, A.K., Ray, D.D., Ghosh, S., 2012. Acaricide resistance status in Indian isolates of *Hyalomma anatolicum*. Exp. Appl. Acarol. 58, 471–481.

Singh, N.K., Jyoti, Abhijit, N., Harkirat, S., 2019. Detection of multi-acaricide resistance in *Rhipicephalus* (*Boophilus*) *microplus* (Acari: ixodidae). Explor. Anim. Med. Res. 9, 24–28.

Singh, N.K., Jyoti, Haque, M., Singh, H., Rath, S.S., Ghosh, S., 2014. A comparative study on cypermethrin resistance in *Rhipicephalus* (*Boophilus*) *microplus* and *Hyalomma anatolicum* from Punjab (India). Ticks Tick. Dis. 5, 90–94.

Stone, B.F., Haydock, K.P., 1962. A method for measuring the acaricide-susceptibility of the cattle tick *Boophilus microplus* (Can.). Bull. Entomol. Res. 53, 563–578.

Takata, M., Misato, S., Ozoe, F., Ozoe, Y., 2020. A point mutation in the *β*-adrenergic-like octopamine receptor: possible association with amitraz resistance. Pest Manag. Sci. 76, 3720–3728.

Temeyer, K.B., 2018. Molecular biology of tick acetylcholinesterases. Front. Biosci. Front. Biosci. (Landmark Ed) 23, 1320–1337.

Van Zee, J.P., Hill, C.A., 2017. Using comparative genomics to decode the genetics of acaricide resistance. Front. Biosci. 23, 889–908.

Temeyer, K.B., Pruett, J.H., Olafson, P.U., 2010. Baculovirus expression, biochemical characterization and organophosphate sensitivity of rBmAChE1, rBmAChE2, and rBmAChE3 of *Rhipicephalus* (*Boophilus*) *microplus*. Vet. Parasitol. 172, 114–121.

Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W., Tirry, L., 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. Insect Biochem. Mol. Biol. 40, 563–572.

Villar, D., Klafke, G.M., Rodríguez-Durán, A., Bossio, F., Miller, R., Pérez de León, A.A., Cortés-Vecino, J.A., Chaparro-Gutiérrez, J.J., 2019. Resistance profile and molecular characterization of pyrethroid resistance in a *Rhipicephalus microplus* strain from Colombia. Med. Vet. Entomol. 34, 105–115.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Peña, A., Horak, I.G., Latif, A.A., Pegram, R.G., Preston, P.M., 2003. Ticks of domestic animals in Africa: a guide to identification of species. Biosci. Reports, Edinburgh, U.K 3–210.

Winston, P.W., Bates, D.H., 1960. Saturated Solutions For the Control of Humidity in Biological Research. Ecology 41, 232–237.

Zeller, H.G., Cornet, J.P., Camicas, J.L., 1994. Experimental transmission of Crimean-Congo hemorrhagic fever virus by West African wild ground-feeding birds to *Hyalomma marginatum rufipes* ticks. Am. J. Trop. Med. Hyg. 50, 676–681.