

Acaricide resistance in ticks from livestock farms in Abu Dhabi, United Arab Emirates

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Original research

ABSTRACT

The resistance status in *Hyalomma* ticks collected from animal farms in three different regions of the Emirate of Abu Dhabi (UAE) was estimated using adult immersion tests with various concentrations of cypermethrin (50ppm, 100 ppm, 200 ppm, 400ppm and 800 ppm). Regression graphs of tick mortality plotted against cypermethrin concentrations were utilized to determine lethal concentrations (LC₅₀ and LC₉₀). The data generated on different life history variables (mortality, egg mass weight, reproductive index, and percent inhibition of oviposition) were used to assess the resistance status. Results revealed level III cypermethrin resistance in *Hyalomma anatolicum* of the Al Hamam region (Al Ain) and level II resistance in the Malaqet region (Al Ain), whereas level I resistance was observed in the other studied locations, except Sweihan (Al Ain) where the tick population was fully susceptible. The LC₉₀ value of susceptible (Sweihan) *H. anatolicum* was 244.87ppm and the discriminating dose (DC) was calculated to be 489.74ppm. The present study is the first report of cypermethrin resistance in ticks of the United Arab Emirates and highlights the need to further monitor resistance evolution during the implementation of tick control programs.

Keywords *Hyalomma* spp.; ticks; resistance; cypermethrin; in vitro bioassay

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Introduction

Ticks constitute a major threat to the livestock industry of the MENA (Middle East and North Africa) affecting the production of milk, meat, wool and hides (FAO, 2020). Productivity losses due to the voracious blood-feeding habit of ticks, injection of toxins, and transmission of pathogens such as *Theileria* spp. or *Rickettsia* spp. (Al-Deeb and Muzaffar 2020) have led to annual economic losses estimated at US 18.7 billion (Dzemo *et al.* 2022; Sungirai *et al.* 2018). These losses can be minimized by treating infected animals with acaricides. However, if poorly supervised, the application of acaricides can result in the emergence of resistance in ticks (FAO 2004; Rodriguez Vivas *et al.* 2007; Sharma *et al.* 2012).

Limited studies have been performed on the ticks and their associated host-parasite interactions in the Middle East region. Commonly available acaricides, like synthetic pyrethroids (cypermethrin and deltamethrin) and formamidines, are used indiscriminately for the control of ticks by livestock owners (Al-Deeb and Muzaffar 2020). In the Al Ain region (UAE), high

tick loads by *Hyalomma dromedarii*, the most prevalent tick species in the region, have been reported on camels (Perveen *et al.* 2020), with up to 102 ticks/animal. To reduce infestations on camels, cypermethrin is the most commonly used acaricide (46.9% of camel owners), followed by diazinon (15.6%), α -cypermethrin (15.6%), fenvalerate (14.1%), and amitraz (Al-Deeb and Muzaffar 2020). *H. dromedarii* ticks were reported to be found on camels in a private farm in Al Ain over the entire year, despite monthly applications of an acaricide (Perveen *et al.* 2020), suggesting either the inadequate use of the acaricide or the development of acaricide resistance.

Diagnosing resistance to acaricides is performed primarily through bioassays, the most common being the adult immersion test (AIT) (Whitnall and Bradford 1947), the larval packet test (LPT) (Stone and Haydock 1962) and the larval immersion test (LIT) (Shaw 1966; Dzemo *et al.* 2022). The current study was undertaken to test for the development of resistance against cypermethrin in *H. anatolicum* in the Al Ain region using *in vitro* AIT bioassays and ticks collected from farms in the three regions of the Emirate of Abu Dhabi.

Material and Methods

Study Area

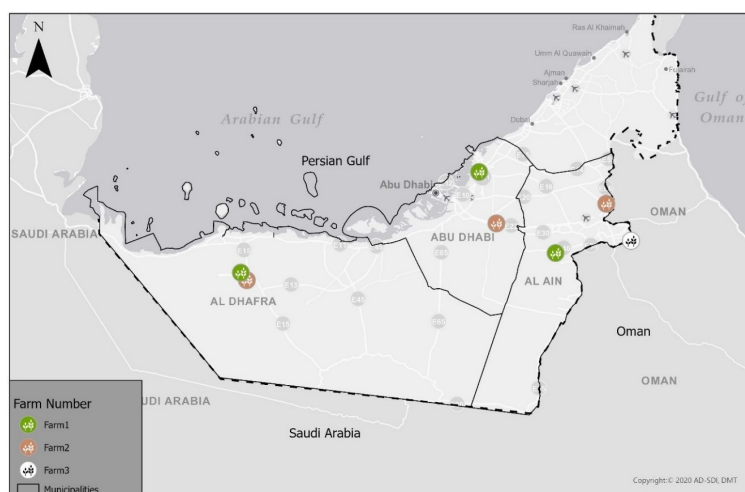


Figure 1 Farm locations within the different regions of the Emirate of Abu Dhabi. Farms are indicated by colored illustrations and the associated information on the farm is presented in Table 1.

Abu Dhabi is located in the west and southwest part of the United Arab Emirates (UAE) along the southern coast of the Persian Gulf (Figure 1). The Emirate's high summer (June to August) temperatures are associated with high relative humidity and winters are relatively mild favouring tick survival. In 2020, a questionnaire was designed to collect data from 15 farm owners in three regions of Abu Dhabi related to the frequency, type, and mode of acaricide treatment adopted and their experiences on the efficacy of commonly used molecules (see supplementary materials). Ticks were then collected from seven of these farms based on questionnaire results (Table 1); we selected farms with mixed farming practices that had tick infestation problems and observed tick treatment failures. *Hyalomma anatolicum* was the most common tick collected from these farms based on morphology (Walker 2003) and was used for resistance studies. Live engorged female ticks were collected from naturally infested livestock in vials, closed with muslin cloth to allow air and moisture exchange, and were brought to the laboratory for experimental assays.

Table 1 Details on each sampled animal farm. See Figure 1 for locations.

Region	Farm	Animal Type (and approximate numbers)		Type of Management*
AbuDhabi	Al-Samha (Farm1)	Camel<10	Sheep>100	Intensive
		Goat>100	cattle <50	
	Al Khatam (Farm2)	Camel<10	Sheep>100	Intensive
		Goat<100		
Al Dhafra	Liwa (Farm1)	Camel<100	Sheep>100	Intensive
		Goat>100	Cattle<10	
	Liwa (Farm2)	Camel<100	Sheep>100	Intensive
		Goat>100		
	Sweiha (Farm1)	Camel<100	Sheep>100	Intensive
		Goat>100	Cattle<50	
AlAin	Al Hamam (Farm2)	Camel<100	Sheep>100	Intensive
		Goat>100		
	Malaqet (Farm3)	Camel<50	Sheep<100	Intensive
		Goat<100	Cattle<10	

*Animals housed indoor in stalls

Adult Immersion Test (AIT)

Adult immersion tests were performed as per the method of Drummond *et al.* (1973) and Singh *et al.* (2014). The AIT uses engorged females that are immersed in solutions made with technical or commercial acaricides and compares oviposition rates between treated and untreated groups. The eggs can likewise be analysed by weight and viability. Female mortality rate can also be evaluated, which reduces the time necessary to obtain results (1–2 weeks) compared to the time required to determine hatchability (5–6 weeks). In our study, a total of 560 engorged adult female ticks of *Hyalomma anatolicum* were used and each drug concentration was replicated thrice using five adults each time along with a control group. Ticks weighing less than 150mg or that were not fully engorged were discarded. Selected fully engorged female ticks were immersed in different concentrations of cypermethrin (50 ppm, 100 ppm, 200 ppm, 400 ppm, 800 ppm) prepared from an aqueous dilution of a commercial preparation of cypermethrin (10.25% EC) for two minutes and then were dried on filter paper before transferring them into individually labelled plastic tubes. Ticks were placed in a constant climate chamber (Memmert HPP) at 28 ± 2 °C and 85 ± 5% relative humidity for a period of 14 days. Tick mortality was recorded on the 14th day post-treatment by observing the loss of mobility and pedal reflex after exposure to light. In this study, ticks which did not oviposit after 14 days were considered as dead as under optimal rearing conditions, the period of oviposition of engorged female ticks of *H. a. anatolicum* was recorded as a range of 7-12days (Anusha, 2014; Akil, M. *et al.*, 2019).

Ticks collected from the same farm were treated on the same day with different drug concentrations along with a control (in distilled water) and data were recorded per farm. Observations on egg laying was performed 7-10 days post-treatment and egg mass was recorded on day 14. The reproductive index (RI) and the percent inhibition of oviposition for each acaricide concentration was calculated using the following formulae (Sharma *et al.* 2012).

$$Reproductive\ index\ (RI) = \frac{egg\ mass\ weight}{engorged\ female\ weight}$$

$$Percent\ inhibition\ of\ oviposition\ (IO\%) = \frac{RI\ control - RI\ treated}{RI\ control} \times 100$$

Statistical Analysis

Dose response data were analyzed by the probit method (Finney 1962) using IBM SPSS Version 28.0.0.0. A Chi-square test was used to compare the observed mortality for each dose with the frequency predicted by the probit function. The LC_{50} and LC_{90} values for cypermethrin were determined by applying regression equations to the probit transformed mortality data.

Results and Discussion

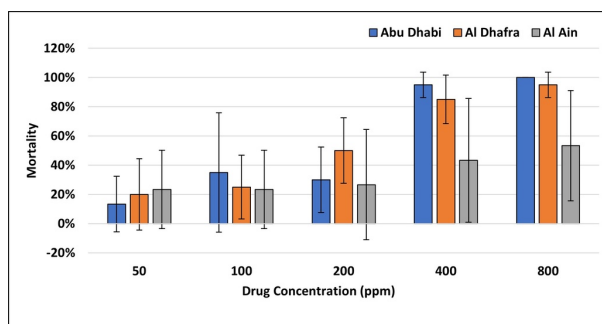


Figure 2 Average tick mortality ($\pm$ SD) to different concentrations of cypermethrin across regions based on three replicate treatments per concentration per farm in Abu Dhabi (n=2 farms), Al Dhafra (n=2 farms), and Al Ain (n=3 farms). The control group showed no mortality.

As outlined above, mixed farms with heavy tick infestations and those for which pyrethroid acaricide treatment failure seemed to regularly occur were selected for the study based on results of the questionnaire (n=7 farms, Table 1). Tick infestations on these farms was observed throughout the year and tick-borne diseases were previously diagnosed, particularly in sheep and cattle. Camels were reared in the same area with sheep and goats, separated only by a small net fence. Keeping animals together in herds and on sandy floors were considered as potential risk factors for the presence of ticks. Indeed, local farms can provide a suitable refuge for ticks because they provide a habitat with relatively high moisture sheltered from the harsh desert environment (Al-Deeb and Muzaffar 2020).

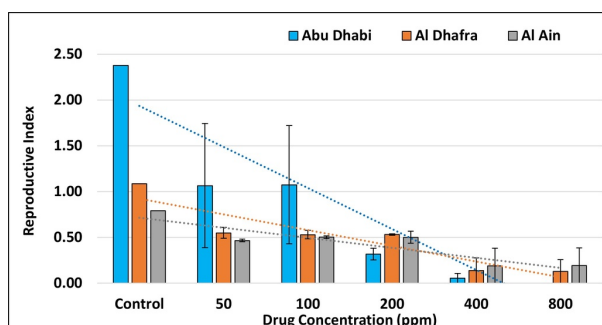


Figure 3 Average tick reproductive index ($\pm$ SD) across regions in relation to different concentrations of cypermethrin based on three replicate treatments per concentration per farm in Abu Dhabi (n=2 farms), Al Dhafra (n=2 farms), and Al Ain (n=3 farms).

Tick populations have an immense potential for rapidly developing resistance due to their biological and behavioral characteristics, and resistance to different active ingredients has

been reported in almost all countries where ticks occur (Alonso-Diaz *et al.* 2006). Synthetic pyrethroids are the most widely and frequently used acaricides to control tick populations. The present study aimed to detect acaricide resistance to the commonly used pyrethroid acaricide cypermethrin in field-collected *Hyalomma anatolicum* ticks from selected farms of the Emirate of Abu Dhabi. A concentration dependent increase in adult tick mortality was observed for cypermethrin in Abu Dhabi and Al Dhafra farms, whereas this relationship was weaker in Al Ain farms (Figure 2). The reproductive index (RI) declined as drug concentration increased (Figure 3), with the strongest decline for ticks from Abu Dhabi farms. In contrast, the inhibition of oviposition increased with the cypermethrin dose (Figure 4) in all farms.

Table 2 Probit transformed mortality data obtained by Adult Immersion tests on *Hyalomma anatolicum* against different concentrations of cypermethrin.

Region	Abu Dhabi	Al Dhafra	Al-Ain		
			Sweihan(Farm1)	AlHamam(Farm2)	Malaqet(Farm3)
Slope*	2.77 ± 0.48	2.19 ± 0.39	1.887±0.68	2.51±1.57	0.98±0.54
χ^2	10.33	2.15	0.75	0.37	2.64
R ²	0.76	0.96	0.75	1	0.88
LC ₅₀ (ppm)	165.85	162.82	51.25	1609.55	939.91
LC ₉₀ (ppm)	481.36	625.61	244.87	5212.84	1651.85
RF Values	3.23	3.17	1	31.4	18.33

*Slope of mortality calculated from mean mortality of ticks to various concentrations of drug

Chisquare value χ^2

R² goodness of fit, LC₅₀ and LC₉₀ calculated from the regression equation

RF resistance factor

Among the three farms in Al Ain region, two farms namely Al Hamam (Farm2) and Malaqet (Farm3), showed low tick mortality at the tested doses and could not produce 100% mortality even at the highest concentration. (Figure 5). The reproductive indices were not affected and the percent inhibition of oviposition was less than 45% (Figures S1 and S2 in Supplementary Materials). These results indicate the acaricide resistance status of the ticks in these farms, with fully susceptible ticks present on farm 1 and very resistant ticks on farms 2 and 3.

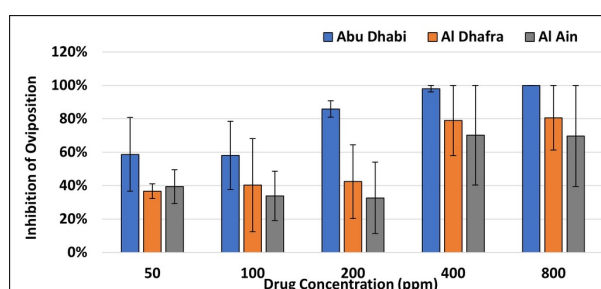


Figure 4 Average percent inhibition of tick oviposition (±SD) across regions in relation to different concentrations of cypermethrin based on three replicate treatments per concentration per farm.

The present study is the first of its kind in this region and throws some light on the resistance status of ticks against a frequently used acaricide. Abu Dhabi and Al Dhafra regions harbour tick populations that are heterogenous in terms of resistance, with LC₉₀ values falling within the discriminating dose reported by earlier studies (Shyma *et al.* 2013). *Hyalomma* ticks from Sweihan (Farm 1 in Al Ain) were found to be susceptible to cypermethrin and the discriminating dose (2xLC₉₀) was calculated as 489.74ppm in the present study.

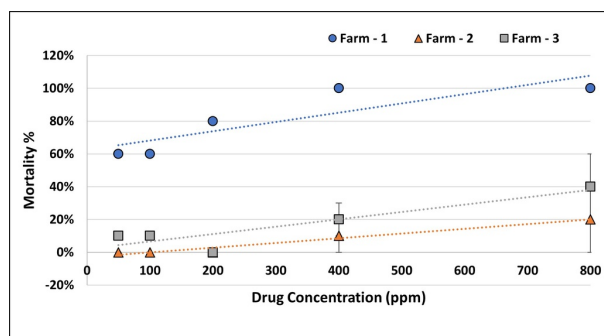


Figure 5 Average mortality of ticks ($\pm$ SD) in the Al Ain region (n= 3 farms) after cypermethrin treatment. Control groups showed no mortality in the three farms.

Singh *et al.* (2014) classified resistance in ticks based on the calculated resistance factor (RF): susceptible ($RF \leq 1.4$), level I resistant ($RF = 1.5-5.0$), level II resistant ($RF = 5.1-25.0$), level III resistant ($RF = 25.1-40$) and level IV resistance ($RF > 40.1$) in reference to a susceptible tick reference strain. Resistance factors (RF) for field tick samples were worked out by the quotient between LC_{50} of field ticks and LC_{50} of susceptible Sweihan *H. anatolicum* (Castro-Janer *et al.*, 2009). Based on the regression analyses of the obtained data with the Sweihan isolate as the susceptible strain (Sharma *et al.* 2012; Shyma *et al.* 2012; Singh *et al.* 2014; Surbhi *et al.* 2018), the resistance factor (RF) was calculated to ascertain the susceptibility and resistant status of the other studied population. The Al Hamam isolates had Level III resistance, Al Malaqet isolates had Level II and the four farms in the Abu Dhabi and Dhafra regions showed Level I resistance (Table 2).

In the current study, the LC_{90} value of susceptible ticks (Sweihan population) by AIT bioassay was 244.87ppm, while it was over 5000ppm in the AlHamam farm of the same region. Much reported work on cypermethrin resistance has been conducted on *Rhipicephalus microplus* and a wide variation in LC_{90} values has been found. Singh *et al.* (2019) found an LC_{90} value of 183.07 ppm for *R. microplus* from the Jalandhar district of Punjab, India. Singh *et al.* (2015) reported level I resistance to cypermethrin in *H. anatolicum* collected from the Gujarat state, India using larval packet test. Sharma *et al.* (2012) found a LC_{50} value of cypermethrin of 138.5 ppm with a 95% confidence interval of 134.5–142.6, while the LC_{95} value was 349.1 ppm with a 95% confidence interval of 323.2–377.0 using a laboratory reared susceptible line of *R. microplus* in Izatnagar, India. This variance among studies has been attributed to differences in the reference tick lines used and in the type of bioassay adopted (AIT or LPT) (FAO 2004; Robertson *et al.* 2006; Castro-Janer *et al.* 2009; Aguirre *et al.* 2000; Jonsson *et al.* 2007; Klafke *et al.* 2017).

This study forms the first report on acaricide resistance in ticks of the UAE. A country specific discriminating concentration (DC = two times the concentration that would kill 99% of ticks susceptible to the molecule) was not previously available for any tick species, hence the generation of baseline data for an abundant tick species is useful for monitoring changes in the resistance status of ticks in this Emirate. Here, we calculated the DC for commercially available cypermethrin as 489.74 ppm for *Hyalomma anatolicum*. This DC can now be used as a screening mechanism because one can test for resistance in small samples from diverse locations (FAO 2004).

The development of resistance in a tick population can depend on factors related to acaricide use, ecological niches and the genus of ticks involved (Kunz and Kemp 1994; Singh *et al.* 2019). A limitation in the present study was the difficulty in obtaining adequate numbers of engorged females for testing, which may affect the reproducibility of AIT values (Jonsson *et al.* 2007). Further studies with higher sample sizes are recommended to obtain more predictive values of

AIT. Similarly, a commercial formulation of cypermethrin was used in the present study as the pure technical grade was unavailable. The presence of proprietary ingredients in commercial formulations can hinder the responses to active ingredients (Shaw 1966) which could alter the results and might be one of the reason for the relatively high LC₉₀ value that we found. Tests based on technical grade cypermethrin would therefore be useful to validate our results. The frequent use of acaricides leading to the emergence of resistant population, as observed in our results, likely explains the presence of ticks on treated animals as reported by Perveen *et al.* 2020. The present study reported only *Hyalomma* spp. ticks in the farms in the region. Based on morphological characteristics, only *Hyalomma anatolicum* (90%) and *H. dromedarii* (10%) were identified (Walker 2003). The practice of rearing camels with sheep and goats and mixing them on common grazing habitats likely increases the risk of ticks shifting among alternative hosts. Moreover, local farms provide suitable environments for ticks, with sand beds in which ticks can burrow a few centimeters below ground and find both favorable microhabitats for egg deposition and refuge from the chemical spraying. Improper use of acaricides in terms of dose, frequency and method of application can thus result in acaricide failure. Based on the data obtained on the emerging problem of tick resistance to chemical acaricides, an alert on good practices for tick control to improve the drug efficacy is recommended, along with the implementation of a program to monitor the evolution of acaricide resistance.

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