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The Potential of Huwa-San TR50 as an Acaricide for Controlling *Hyalomma dromedarii* (Koch) and *Hyalomma impeltatum* (Schulze & Schlottke) (Acari: Ixodidae)

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ABSTRACT

In this study, an alternative control method was developed for camel tick (*Hyalomma dromedarii* Koch) and cattle tick (*Hyalomma impeltatum* Schulze & Schlottke), to replace the widely used traditional acaricides. Huwa-San TR50 is a formulation of hydrogen peroxide, stabilized by the addition of a small quantity of silver and is extensively used as a disinfectant. Both tick species were susceptible to certain concentrations of Huwa-San TR50: 4,000, 8,000, 10,000, 12,000 and 14,000 ppm. A marked reduction in the movement of both tick species was observed 24h of applying Huwa-San TR50, at 10,000 ppm and above. This study recorded an increase in percentage mortality with increasing concentrations of Huwa-San TR50 and this resulted in mortalities of 66.5% for *H. dromedarii* and 76.5% for *H. impeltatum* after 24 h of direct exposure (direct spray treatment) whereas in the dipping treatment, the mortality was 60% and 70% for *H. dromedarii* and *H. impeltatum* at 14,000 ppm of Huwa-San TR50. Huwa-San TR50 was found to be moderately effective in killing both tick species. Therefore, it can be used to develop a new and safe strategy for controlling ticks, so as to produce dairy and meat products with minimal residual contamination.

INTRODUCTION

The camel tick (*Hyalomma dromedarii* Koch), is distributed wherever camels are found in the desert, semi-desert and steppes (Hoogstraal *et al*., 1981). *H. dromedarii* is mainly hosted by camels and cattle but it prefers camels while the adults of horses, sheep, donkeys, buffaloes and mules are less common hosts (Montasser, 2006). Immature *H. dromedarii* is also associated with camels but can parasitize a wide variety of birds, rodents, hares and hedgehogs (Hoogstraal, 1956).

H. dromedarii is a carrier of many life-threatening viral diseases such as the Crimean-Congo hemorrhagic fever (CCHF) virus (Hoogstraal, 1979; Rodringuez *et al*., 1997), Kadam virus (Wood *et al*., 1982), Dera Ghazi Khan virus, Dhori virus (Hoogstraal *et al*., 1981), Quaranfil virus (Converse and Moussa, 1982), Q fever (*Coxiellaburnetii*) (Bazlikova *et al*., 1984), spotted fever rickettsia (*Rickettsia rickettsia*) (Lange *et al*., 1992) and theileriosis of camel (*Theileria camelensis*) and cattle (*Theileria annulata*) (Hoogstraal *et al*., 1981).

The cattle tick (*Hyalomma impeltatum* Schulze and Schlottke) is widely distributed in Saudi Arabia, the Near East and south-western part of Central Asia and Africa north of the equator (Apanaskevich and Horak, 2009). Immature *H. impeltatum* parasitizes birds, leporids, rodents, and lizards (Apanaskevich and Horak, 2009). It is also considered to be a vector of the CCHF virus (Dohm *et al*., 1996). In addition, El-Azazy *et al*. (2001) found that *H. impeltatum* was associated with the majority of Theileria-infested sheep in Saudi Arabia. Malignant theileriosis, caused by *Theileriahirci,* is an economically important disease in small ruminants, especially in sheep (Uilenberg, 1997).

Tick control is mainly based on the direct application or injecting (*e.g.* ivermectin) of acaricides to animals. The following acaricides have been extensively used and commonly recommended for use in tick control, organophosphates (*e.g.* diazinon, dioxathion and coumaphos), carbamates (*e.g.* carbaryl), pyrethroids (*e.g.* deltamethrin, flumethrin, permethrin and decamethrin), and amidines (*e.g.* amitraz) (De Meneghi *et al*., 2016).

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Several studies have focused on testing the efficacy of a wide range of acaricides against ticks (*in vitro*, *in vivo* and both). For example, El-Azazy and Lucas (1996) tested the efficacy of flumethrin (a synthetic pyrethroid) on the fertility of engorged females of *H. dromedarii*. Petros *et al*. (2015) examined the acaricidal efficacy of amitraz and diazinon *in vitro* and *in vivo* against *Rhipicephalus pulchellus* and *H. dromedarii* infecting camels in Jigjiga, Eastern Ethiopia. Al-Rajhy *et al*. (2003) reported the efficacy results of cardiac glycosides, azadrichtin and Neem oil against *H. dromedarii* in Saudia Arabia. Straten and Jongejan (1993) evaluated the efficacy of ivermectin against *H. dromedarii*in Sinai, Egypt. Also, Constantin *et al*. (2012) tested the efficacy of four different acaricides namely ivermectin, deltamethrin, diazinon and amitraz against *H. impeltatum*.

In the last decade, health concerns were highlighted in several studies, as a result of the presence of residues of diazinon in the milk of cows (Hastie, 1963; Mathysse and Fisk, 1968; Leschchev *et al*., 1972; Bull and McDougall, 1974) and sheep (Formica, 1973). However, diazinon has less prolonged residual activity as compared with organophosphates which are not recommended for use in lactating cows (De Meneghi *et al*., 2016). Control strategies involving inappropriate use of acaricides or incorrect concentrations may result in unacceptable residues. This is partly responsible for the development of tick resistance to all available acaricides on the market thereby resulting in the failure of tick control programs as well as environmental contamination.

On the other hand, several studies have focused on finding an alternative strategy (*e.g.* a biological control agent) for managing tick infestation. Among the biological control candidates studied to date are entomopathogenic nematodes (Zhioua *et al*., 1995; Hill, 1998), a wasp parasitoid (Hu *et al*., 1993; Stafford *et al*., 1996; Knipling and Steelman, 2000), vertebrate predators (Duffy *et al*., 1992; Ostfeld and Lewis, 1999), entomopathogenic fungi (Zhioua *et al*., 1997, 1999a) and entomopathogenic bacteria (Zhioua *et al*., 1999b). Among these biological candidates, entomopathogenic fungus *Metarhizium anisopliae* Metschnikoff was highly pathogenic to engorged larvae and engorged adult female *Ixodes scapularis* (Zhioua *et al*., 1997; Benjamin *et al*., 2002). *M. anisopliae* was also considered as a potential acaricide as it showed high efficacy against organophosphate-resistant strains and susceptible strains of the *Boophilus microplus* tick (Fernández-Ruvalcaba *et al*., 2005).

Infestation by a large number of ticks can cause serious problems and pose a challenge to animal keepers in both developed and developing countries. Tick infestation causes harm to animal hosts either directly or indirectly. Directly, ticks cause blood loss and injuries which can provide a route for secondary infection (Constantin *et al*., 2012). Indirectly, ticks are also responsible for economic losses as they play a critical role in the transmission of many vector borne diseases and toxins to domestic animals and man (Karesh *et al*., 2005; Pietzsch *et al*., 2006). As a result of international trade, ticks also spread pathogens in new geographical areas (Gonzalez-Acuna, 2005; Soorae *et al*., 2008). This summarizes and highlights the economic and health importance of ticks and emphasizes the need for researchers to find an alternative method of tick control, so as to gain highly effective protection with minimal impact on environmental and public health.

Huwa-San TR50 is widely used as a disinfectant and was developed over twenty years ago (www.huwasan.com). It is a formulation of hydrogen peroxide, stabilized by the addition of a small quantity of silver (www.huwasan.com). According to our literature review, a few published reports are available on tick control and Huwa-San TR50 has never been used for the control of *H. dromedarii* and *H. impeltatum*. Alhewairini (2017) was the first to successfully use Huwa-San TR50 as an insecticide for controlling cotton aphids (*Aphis gossypii* Glover) without any significant effects on honeybees (*Apis mellifera lamarckii*) and seven-spot ladybird beetles (*Coccinella septempunctata*). In addition, Alhewairini and Al-Azazzy (2017a, b) were the first to report that Huwa-San TR50 can be successfully used as an acaricide in killing two spotted spider mites (*Tetranychus urticae* Koch) with minimal effects on its associated predatory mite, (*Neosiulus cucumeris*) and *Varroa* mite (*Varroa jacobsoni* Oudemans).

Huwa-San TR50 potentially has several advantages that make it reliable and safe, such as its high efficacy even at low concentrations, being effective under a wide range of temperatures up to boiling point, being gentle to the skin, having long term effectiveness, being biodegradable, causing no build-up of resistance by microorganisms, as well as being non-toxic to humans, colorless, tasteless and odorless (www.huwasan.com).

The main objective of this study was to evaluate the potential efficacy of Huwa-San TR50 against *H. dromedarii* and *H. impeltatum*.

MATERIALS AND METHODS

Ticks

Attached and non-treated adults (males and fully engorged females) of *H. dromedarii* were randomly collected from a heavily infested camel farm (*Camelus dromedarius*) in Al-Badaya city, Al-Qassim, Saudi Arabia. Adults (males and fully engorged females) of *H.*

impeltatum were randomly collected from infested cows (*Bostaurus*) at the Experimental Research Station, Qassim University, Buraidah, Al-Qassim, Saudi Arabia.

Chemicals and solutions

Huwa-San TR50 was obtained as a gift from Ghatafan Company in Onaizah (retailer agent). The stock solution of Huwa-San TR50 (500,000 ppm) was diluted with distilled water to give a concentration range between 4,000 to 14,000 ppm. The experiments were conducted under laboratory conditions, throughout April 2017.

Experimental protocol

All ticks were collected from 20 camels and 25 cows. The average tick number was between 20 and 42 ticks/camel and between 15 and 24 ticks/cow. Both ticks were collected at 7 am. In order to minimize damage to the mouthparts and cuticle, the ticks were manipulated by rotating for easy removal with a pair of soft forceps. All treated ticks were examined under a stereomicroscope and ticks with damaged cuticle or mouthparts were excluded. The collection of ticks was arranged in a randomized complete block design. Collected ticks were placed in a clean plastic container and immediately transported to the laboratory (25±2ºC and 75% relative humidity) at the Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University for bioassay. The ticks were identified according to Hoogstraal *et al*. (1981). Groups of 30 ticks of equal size were used and each treatment group was placed in a 14cm Petri dish. Five concentrations of Huwa-San TR50 were used: 4,000, 8,000, 10,000, 12,000 and 14,000 ppm. Controls were exposed to distilled water. Each treatment was replicated 4 times. Both species of tick were exposed to Huwa-San TR50 either by direct spray or dipping. For direct spray treatment the five concentrations of Huwa-San TR50 and the control were directly sprayed onto both tick species using a small knapsack sprayer (1L). Dipping treatment was carried out by transferring ticks to labelled 50ml tube and adding 25ml of the five concentrations of Huwa-San TR50 or the control treatment. The tubes were shaken for one minute after which the contents of the tube was sieved to remove the solution.

After both treatments ticks were transferred to new Petri dishes and assessed for mortality after 24 h. Immobile ticks that did not respond to mechanical stimulation were incubated at 28^{o}C for 5 days and observed daily to determine final mortality values.

Statistical analysis

The mortalities of *H. dromedarii* and *H. impeltatum* were calculated manually by direct observation. Mortality is the average number of dead ticks divided by the initial number in the group expressed as a percentage. Thereafter, the average of the obtained mortality data and mortality percentages were calculated using the Microsoft Excel Program. Statistically, all variables of the obtained data were analyzed using one-way analysis of variance (ANOVA). The IC_{50} values for the effects of Huwa-San TR50 on both *H. dromedarii* and *H. impeltatum* were calculated using Graphpad Prism 8 to determine the significant difference between two methods of application (direct spray and dipping in solution) among one species and between two species.

RESULTS

The results showed that both *H. dromedarii* and *H. impeltatum* were susceptible to a concentration of Huwa-San TR50.

Table I.- Effect of five concentrations of Huwa-San TR50 on camel tick, *Hyalomma dromedarii* Koch, under laboratory conditions.

Concentration (ppm)	No. of ticks *H. dromedarii*					
	Direct spray			Dipping		
	Avg. pre-spray count	Avg. post-spray count*	% Mortality**	Avg. pre-dipping count	Avg. post-dipping count*	% Mortality**
Control	30	30	0.0 a	30	30	0.0 a
4,000	30	20	33.33 b	30	21	30 b
8,000	30	18	40 c	30	19	36.66 c
10,000	30	15	50 d	30	16	46.66 d
12,000	30	12	60 e	30	13	56.66 e
14,000	30	10	66.66 e	30	12	60 e

*Counts made 24h post treatment. ** Mortality values calculated using the Microsoft Excel Program. Different letters in the vertical columns denote significant difference, (F-test, $P < 0.05$, $P < 0.01$).

Table II.- Effect of five concentrations of Huwa-San TR50 on cattle tick, *Hyalomma impeltatum* Schulze & Schlottke, under laboratory conditions.

Concentration (ppm)	No. of ticks *H. impeltatum*					
	Direct spray			Dipping		
	Avg. pre-spray count	Avg. post-spray count*	% Mortality**	Avg. pre-dipping count	Avg. post-dipping count*	% Mortality**
Control	30	30	0.0 a	30	30	0.0 a
4,000	30	17	43 b	30	18	40 b
8,000	30	16	46 c	30	17	43 c
10,000	30	13	56 d	30	13	56 d
12,000	30	9	70 e	30	10	66 e
14,000	30	7	76 e	30	9	70 e

*Counts made 24h post treatment. ** Mortality values calculated using the Microsoft Excel Program. Different letters in the vertical column denote significant difference, (F-test, $P < 0.05$, $P < 0.01$).

In the direct spray treatment trial, the percentage mortality was 33.33, 40, 50, 60 and 66.5% for *H. dromedarii* and 43.33, 46.5, 56.5, 70 and 76.5% for *H. impeltatum* at 4,000, 8,000, 1,0000, 12,000 and 14,000 ppm of Huwa-San TR50 after 24h of treatment, respectively (Tables I and II). In the dipping treatment trial, the mortality percentages were 30, 36.5, 46.5, 56.5, and 60% for *H. dromedarii* Koch and 40, 43.33, 56.5, 66.5 and 70% for *H. impeltatum* at 4,000, 8,000, 1,0000, 12,000 and 14,000 ppm of Huwa-San TR50 after 24 h of treatment, respectively (Tables I and II). In either treatment trials (direct spray and dipping in solution), there was no significant difference between 12,000 and 14,000 ppm of Huwa-San TR50, on the mortality of both tick species.

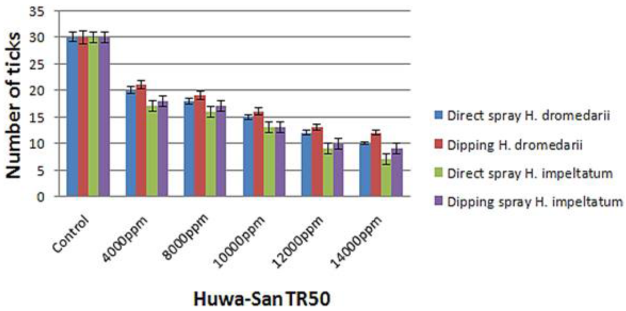

Fig. 1. Comparison of the average effects of Huwa-San TR50 on the mortality of camel ticks (*H. dromedarii* Koch) and cattle ticks (*H. impeltatum* Schulze & Schlottke) under laboratory conditions after 24h of exposure, expressed as a percentage of the control mortality in distilled water. Each bar is the mean ± SEM of 4 replicates of 30 ticks each.

The obtained results demonstrated that Huwa-San TR50 produced a serious malformation to the cuticle of *H. dromedarii* and *H. impeltatum* which is positively related to the concentrations of Huwa-San TR50. All dead ticks were found with a detectable cuticle malformation (Figs. 1 and 2). The death of both tick species may be due to the cuticle malformation caused by exposure to Huwa-San TR50 but it is still unknown if Huwa-San TR50 can cause other internal organ damage. The cuticle malformation of *H. impeltatum* was apparently stronger than that of *H. dromedarii* as its cuticle was almost burned down (Figs. 2 and 3), although they are both species of hard-bodied ticks belonging to the same family Ixodidae.

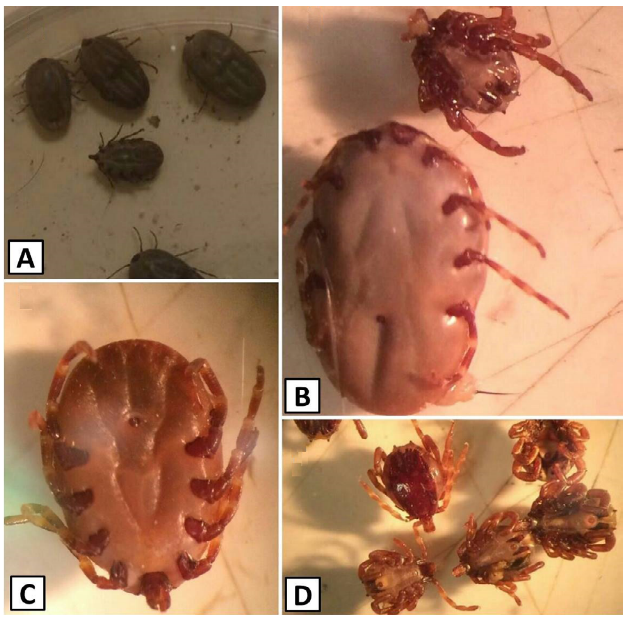

Fig. 2. Camel tick, (*Hyalomma dromedarii* Koch) treated with Huwa-San TR50 under laboratory conditions. Picture A shows non-treated *H. dromedarii* (control), Picture B, Picture C and Picture D show the cuticle damage of *H. dromedarii* treated with 1000, 12000 and 14000 ppm of Huwa-San TR50, respectively. Pictures were taken after 24 h of direct spray treatment.

Fig. 3. Cattle tick, (*Hyalomma impeltatum* Schulze & Schlottke) treated with Huwa-San TR50 under laboratory conditions. Picture A shows non-treated *H. impeltatum* (control), Picture B, Picture C and Picture D show cuticle damage of *H. impeltatum* treated with 1000, 12000 and 14000 ppm of Huwa-San TR50, respectively. Pictures were taken after 24 h of direct spray treatment.

Statistically, the difference between the two treatment trials on the IC_{50} values (direct spray and dipping in solution) was significant (P = 0.031) on *H. dromedarii* and insignificant (P = 0.349) on *H. impeltatum* whereas the difference between two species was insignificant (P = 0.176) for direct spray treatment and significant for dipping (P = 0.002) in solution treatment after 24h of exposure to five concentrations of Huwa-San TR50, including the control (distilled water) (using F-test in Graphpad Prism 8) (Fig. 1; Table III).

Table III.- The IC_{50} values for the effects of Huwa-San TR50 on both camel tick (*Hyalomma dromedarii* Koch) and cattle tick (*Hyalomma impeltatum* Schulze & Schlottke) under laboratory conditions.

Treatments	IC_{50} (ppm) of Huwa-San TR50 (95% CI)	p-value*
H. dromedarii (direct spray)	9208 (7927 to 10653)	P = 0.031
H. dromedarii (dipping)	11314 (9833 to 13727)	
H. impeltatum (direct spray)	6803 (4756 to 8416)	P = 0.349
H. impeltatum (dipping)	7770 (6011 to 9373)	

*Calculated by using F-test in Graphpad Prism 8.

Interestingly, Huwa-San TR50 can cause paralysis immediately after its application, especially at 10,000 ppm and above, since both tick species were unable to move. At lower concentrations of Huwa-San TR50 (4,000 and 8,000 ppm), both tick species showed abnormal movement when compared with the control (distilled water). In addition, male ticks were found to be significantly sensitive to Huwa-San TR50 as compared with engorged adult females.

DISCUSSION

In different localities, ticks are considered the most economically important livestock ectoparasite, as they can cause huge economic losses worldwide. Many health and environmental concerns have arisen regarding toxicity related problems and the growing incidences of tick resistance to available and recommended acaricides. Furthermore, tick infestation is not tolerated by herd owners, hence they might exceed the recommended dose of acaricides to achieve the highest tick mortality. Such an attitude is not only harmful to treated animals but also harmful to man, domestic animals and the environment.

The obtained results showed that Huwa-San TR50 can significantly kill *H. dromedarii* and *H. impeltatum* compared with the control (distilled water) with an observable malformation on their cuticle which is positively related to the concentrations of Huwa-San TR50. This finding is consistent with the results of previous studies conducted by Alhewairini (2017) and Alhewairini and Al-Azazzy (2017a, b). All dead ticks were found with a detectable cuticle malformation (Figs. 2 and 3). Therefore, further investigations are required to understand the mode of action of Huwa-San TR50 on ticks, since this cuticle malformation has never been recorded after the application of many acaricides which are extensively used in tick control.

Petros *et al.* (2015) found that the mortality percentages of *H. dromedarii* were 96, 100% and 92, 100% after 24h of exposure to the recommended and double recommended rates of amitraz and diazinon, respectively. It can be argued here that the residual quantity depends on the concentration and frequency of application. Nevertheless, exceeding the recommended levels might successfully achieve tick mortality but this would increase the residual effects potential in dairy or meat products.

Constantin *et al.* (2012) pointed out that the mortality of *H. impeltatum* was 72, 79, 87 and 90 after one day of exposure to the recommended dose of ivermectin, deltamethrin, diazinon and amitraz, respectively. They also found that 100% mortality was achieved after 5 days for deltamethrin and 7 days for diazinon and amitraz and no reinfection was registered at 21 days after treatment.

In the case of biological control, Fernández-Ruvalcaba *et al*. (2005) found that the mortality following exposure to 10^8 spores/ml of *M. anisopliae* was 100% in both OP-resistant strains and susceptible strains of the *B. microplus* tick. Moreover, 100% tick mortality of engorged adult female, *I. scapularis* was achieved using *M. anisopliae* at a concentration 10^7 spores/ml (Zhioua *et al*., 1997).

In comparison with the obtained results, maximum mortalities of 66.5% for *H. dromedarii* and 76.5% for *H. impeltatum,* were achieved at 14,000 ppm of Huwa-San TR50 after 24 h of exposure. Clearly, Huwa-San TR50 showed less mortality percentages compared with available acaricides and other microbial control agents. However, higher concentrations of Huwa-San TR50 might be more effective against both tick species but it is beyond this approach, as its environmental impacts and residues must be justified. Nevertheless, Huwa-San TR50 can be considered as a promising material compared with available and recommended acaricides for use in tick control. It would be valuable to test the efficacy of Huwa-San TR50 against resistant ticks as well as the toxicity of Huwa-San TR50 to biological control agents which showed high pathogenicity to ticks.

Amitraz has several properties which make it an excellent detaching agent, including the ability to eliminate ticks from infested animals (Mekonnen, 2001; Natala *et al*., 2005). It is still unknown whether Huwa-San TR50 can provide properties similar to that of amitraz, which would be of interest to further test in the future. Ticks exposed to Huwa-San TR50 did not show any recovery as compared with resistant ticks exposed to the commonly used conventional acaricides. Therefore, it can be assumed after exposure to Huwa-San TR50, it will be difficult for the population of ticks to be sustained. In addition, a higher concentration of Huwa-San TR50 might be more effective against both tick species but its impacts must be justified.

Like other conventional acaricidal agents, Huwa-San TR50 has been found to be feasible for use as an acaricidal agent in controlling ticks; as it was found that Huwa-San TR50 effectively killed two spotted spider mites (*T. urticae*) and *Varroa* mite (*V. jacobsoni*) (Alhewairini and Al-Azazzy, 2017a, b). Huwa-San TR50 is also cheaper than other available conventional acaricides that have been extensively used for controlling ticks such as amitraz and diazinon and has a lower acute toxicity of Huwa-San TR50 on rabbits (dermal LD_{50} > 4,000 mg/kg, 50%H_2O_2) and rats (oral LD_{50} > 500mg, 50% H_2O_2) (Chemi, 2013) compared with amitraz (rabbits dermal LD_{50} = 200 mg/kg and rats oral LD_{50} = 523 – 800mg/kg) (USEPA, 1987) and diazinon (rabbits dermal LD_{50} = 2,000 mg/kg and rats oral LD_{50} = 66 – 635 and 96 – 967mg/kg for females and males, respectively) (USEPA, 1986). Clearly, Huwa-San TR50 has the lowest dermal toxicity and is utilized for tick control. However, Huwa-San TR50 is very toxic to fishes (LC_{50} = 16.4mg/L (96 h of exposure)) (Chemi, 2013); therefore, much care must be taken when applying Huwa-San TR50 around water bodies.

CONCLUSION

This study has provided a new and novel approach which confirms the potential of using Huwa-San TR50 as an acaricide in veterinary purposes, as Huwa-San TR50 showed high efficacy in killing *H. dromedarii* and *H. impeltatum*. Clearly, Huwa-San TR50 produced a serious malformation on the tick cuticle and this was seen on both tick species tested in this study. Therefore, it was hypothesized that the cuticle malformation resulted in the death of both tick species as there is no evidence in the literature which explains this finding. Therefore, further studies on the mode of action of Huwa-San TR50 on the cuticle of ticks is highly recommended to understand how and why it can kill both tick species. This study has helped in the development of a new and safe strategy for controlling ticks, so as to minimize the residual effects in dairy and meat products.

Further investigations would be very valuable regarding the application of Huwa-San TR50 against other tick species, its effect on other stages of ticks including eggs and its side effects on animals. On the other hand, as Huwa-San TR50 has mainly been used as a bacterial and fungal killer, it would also be very interesting to investigate its efficacy against the bacterial and fungal infections that resulted upon tick infestation.

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Statement of conflict of interest

The authors declare that there is no conflict of interest.

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