Original Article

The effects of water submersion on cattle ticks

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Abstract

Rhipicephalus (*Boophilus*) *microplus* is an important vector transmitting hemoparasites in cattle and is, therefore, the cause of significant loss to cattle production. The control of ticks with synthetic acaricides is partially successful. However, parasite resistance to these compounds has been reported. This study aimed to evaluate the effects of water submersion on cattle ticks. Ticks were divided into three weight classes [small (3-10 mg), medium (10-30 mg) and large (>70 mg)]. All ticks in the small weight class died after immersion in water for as little as 5 mins. Some ticks in the medium weight class died after immersion for 10 and 15 mins but none in the largest weight class died at any time point. Entry of water into the body of the small ticks might be via the openings of the stigmata. In conclusion, water submersion is an alternative way of controlling cattle ticks. This simple technique can be applied to production animals or pets to control ticks cheaply and safely.

Keywords: Cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, Simple technique, Tick control

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Introduction

Ticks suck the blood of animals and transmit several important pathogens such as *Anaplasma marginale, Babesia bigemina* and *Babesia bovis* (Dalgliesh and Stewart, 1983; Guerrero *et al*., 2007; Howell *et al*., 2007). Ticks cause economic impact on cattle production. Heavy tick infestation can cause reduced milk production and lower live-weight gain in dairy and beef cattle (Sutherst *et al*., 1983; Jonsson *et al*., 1998). The cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, is the most widely distributed tick in the world. It is widespread and common in Thailand and transmits both *Babesia* and *Ananplasma* locally. Tick-borne disease causes economic loss estimated at US$ dollar 50 million to improve the genetic potential for productivity by purchasing exotic cattle (Chansiri, 1997).

Tick controls commonly use chemical acaricides. Use of chemical control can lead to the development of acaricide-resistant ticks and the presence of chemical residues in the environment (Van Zwieten *et al*., 2003; George *et al*., 2004; Bandara and Karunaratne, 2017). Buffaloes exhibit a much lower prevalence of tick infestation than do cattle, even where both bovines co-occur (Sajid *et al*., 2009; Jawale *et al*., 2012; Khan *et al*., 2013; Rehman *et al*., 2017). Buffaloes spend much time immersed in water, whereas cattle do not. This suggests a simple technique to kill ticks: water immersion. This cheap and safe method eliminates the need for chemical acaricides, thus avoiding all downstream problems caused by these chemicals.

Materials and Methods

Collection of cattle ticks: Adult female *Rhipicephalus* (*Boophilus*) *microplus* were collected from infested cows at a village in Khok Chan Sub-district, Trakan Phuet Phon District of Ubon Ratchathani Province, Northeast Thailand (15°29'35"N, 105°6'59"E) as shown in Fig. 1. Ticks were stored in 50 ml tubes, covered with gauze and transported to the laboratory. All the ticks were washed with distilled water, blotted using tissue paper and rested for 24 h. Ticks were identified under a stereomicroscope according to morphological criteria (Soulsby, 1982; Walker *et al*., 2003). Actively moving ticks (n=378) were selected.

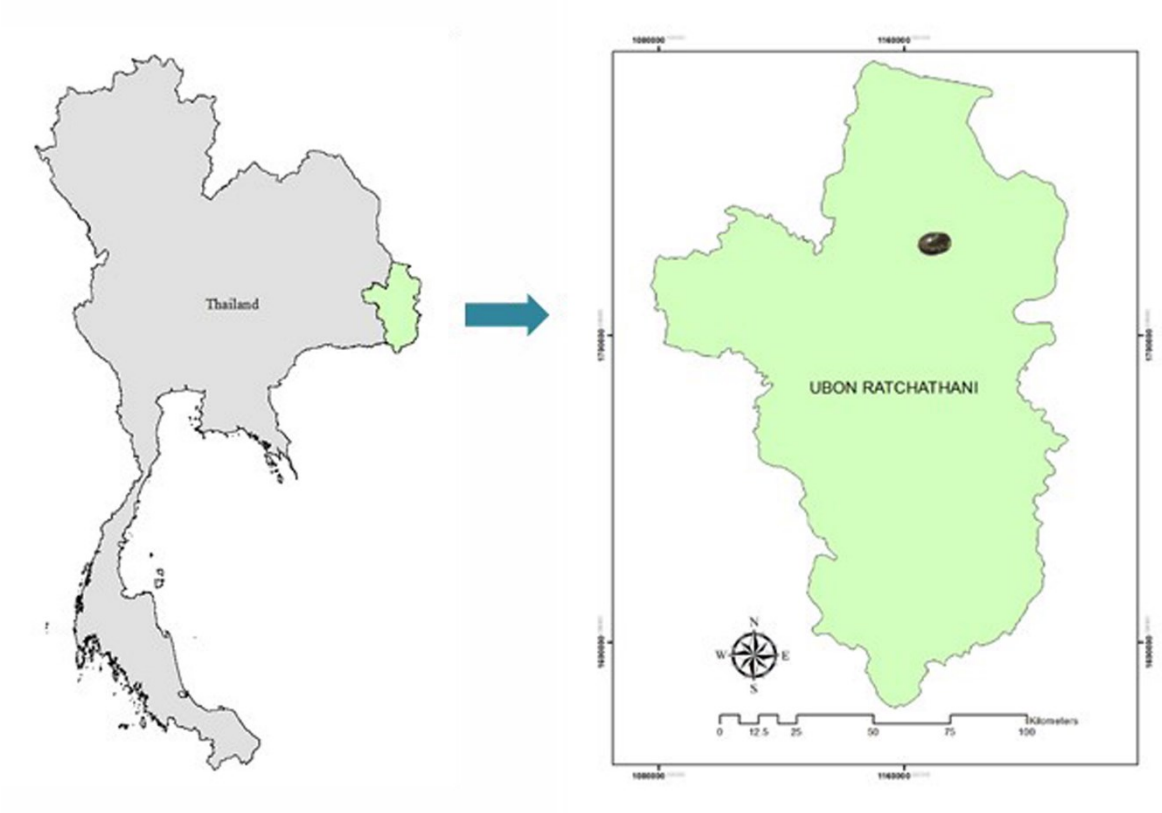

Figure 1 The location of Khok Chan Sub-district, Trakan Phuet Phon District of Ubon Ratchathani Province, Northeast Thailand.

Effects of immersion in water on ticks: Ticks were clarified by bodyweight. Feeding ticks had a bodyweight range from 1.55 to 30 mg, and the engorged tick had a bodyweight range from 30 to 250 mg (Wharton and Utech, 1970; Hadi and Adventini, 2015; Senbill *et al*., 2018). Ticks were weighed to the nearest 0.1 mg using a digital balance (Sartorius) and classified into three weight classes: i) feeding ticks (3-10 mg), ticks not able to produce eggs (Lew-Tabor *et al*., 2018). ii) feeding ticks (10-30 mg), ticks able to produce eggs but a low egg mass. iii) engorged ticks (over 70 mg), ticks needing energy for utilization in the synthesis of yolk proteins, vitellogenins (Kopáček *et al*., 2018). Note that no ticks were found in the weight range 30-70 mg. Ticks in each weight class were separated into 5 groups: i) normal control, ticks were not submerged in distilled water (DW); ii) ticks were transferred with a paintbrush to a distilled water-filled Petri dish and submerged for 5 mins (5 mins DW); iii)ticks were submerged in DW for 10 mins (10 mins DW); iv) ticks were submerged in DW for 15 mins (15 mins DW); and v) ticks were transferred with a

paintbrush to the surface film of the water and allowed to float for 15 mins (15 mins DW float). Each experimental group consisted of 10 individuals, where possible (see Table 1 for details). An extra three individuals were included for the water-penetration experiment (see below).

Preparation of the positive control: Amitraz was purchased from Ecto-tak®, Thailand. Prior to use, 2 ml of 12.5% w/v emulsifiable concentrate amitraz-based compound was added to 1 l of distilled water and stirred well to provide the working solution. From each size class, an additional group of ticks was used. These were submerged in amitraz working solution for 5 mins (Table 1).

Table 1 Mortality of adult female *Rhipicephalus* (*Boophilus*) *microplus* in each experimental group

Size class	Small		Medium		Large	
Treatment	**no. x replicates**	**Mortality[a]**	**no. x replicates**	**Mortality[a]**	**no. x replicates**	**Mortality[a]**
i normal control	n=10x3	0 (0)	n=10x3	0 (0)	n=5x2	0 (0)
ii 5 mins DW	n=10x3	100 (30)	n=10x3	3.33 (1)	n=5x2	0 (0)
iii 10 mins DW	n=10x3	100 (30)	n=10x3	13.33 (4)	n=5x2	0 (0)
iv 15 mins DW	n=10x3	100 (30)	n=10x3	10 (3)	n=5x2	0 (0)
v 15 mins DW (float)	n=10x3	0 (0)	n=10x3	0 (0)	n=5x2	0 (0)
vi 5 mins amitraz	n=13	100 (13)	n=10	90 (9)	n=5	0 (0)

[a] Percentage of ticks that died (number)

Assessment of tick mortality: In all the above experiments, ticks were tested in Petri dishes (9.5 cm diameter, 1.5 cm high) containing 50 ml of distilled water (or amitraz for the positive control). This is a modification of the method used by Politi *et al.*, (2012). After each experiment, ticks were blotted dry on tissue paper, then transferred to new Petri dishes covered with wire mesh. Ticks were confined within individual chambers within the dish. Petri dishes were maintained at 27-28 °C, 70-80 % RH and 12/12 h photoperiod (Politi *et al*., 2012).

Assessment of tick survival: The numbers of dead and live ticks were counted under a stereomicroscope every day for 15 days. Four criteria were used to evaluate tick survival: visible movement of the Malpighian tubules under stereomicroscope, reflex movement of legs after touching with a paintbrush, cuticle color and egg-laying. Each tick was observed for 5 mins. This is modified from the method of Pirali-Kheirabadi and Da Silva (2011).

Assessment of egg production: Alive ticks in the two larger size classes (those in the smallest size class were still immature) continued to lay eggs after treatment with DW or amitraz. After 15 days, eggs were collected for each tick using a paintbrush. Eggs laid by each tick were weighed to the nearest 0.1 mg using an analytical balance (Sartorius).

Measurement of stigmata and body area of ticks: Ticks have a tracheal system opening to the outside via a pair of stigmata on the ventrolateral surface. Gas exchange between the environment and the tracheal system occurs via the stigmata (Woolley, 1972). The stigmata and body surface area of ticks were measured under a stereomicroscope. Ticks are ellipsoidal in shape. The surface area of an ellipsoid is approximated by (4/3) πabc where a is the radius along the x-axis, b is the radius along the y-axis and c is the radius along the z-axis. Openings of stigmata are elliptical in shape, with an area calculated by a longer radius x a shorter radius x π. The ratio of stigmata area and body area was also calculated and expressed as a percentage (a/b) x 100 (where a and b refer to the stigmata area and body area in mm^2 and mm^3, respectively).

Detection of water entering the tick: Possible routes of entry of water into the body of a submerged tick are the mouthparts and the stigmata. We wished to know whether water could enter submerged ticks by either of these routes and perhaps be the cause of death. Three ticks in each experimental group were incubated with propidium iodide (PI) solution (10 mg/ml in DW) for 15 mins (the maximum duration used in our experiments) in a dark room. Ticks were cut with a scalpel (horizontal longitudinal plane) and photographed using a confocal laser scanning microscope (Carl Zeiss; LSM 800) and a fluorescence microscope (Nikon; Ni-U).

Ethics statement: All protocols were approved by the Khon Kaen University Animal Ethics Committee (ACUC-KKU-19/2559).

Data analysis: Mortality data was analyzed using log-rank tests on the cumulative tick death and median survival for each treatment was determined, with 95% confidence interval (CI) in the Stata version 10.1. Egg production of individual ticks in each group was compared using a non-parametric Kruskal-Wallis test and Mann-Whitney U test, (SPSS software version 16). A value of $P < 0.05$ was considered statistically significant.

Results

The effects of distilled water on the mortality of adult female ticks

Ticks in the smallest weight class (3-10 mg): All ticks in this weight class submerged in DW subsequently died, even if immersed for only 5 mins (Table 1). Mortality was significantly higher ($P < 0.001$) in experimental groups ii, iii, iv and vi relative to those in the larger weight classes (Fig. 2A). Fifty percent of ticks died by day 4 (95% CI: 4-7) following immersion in experimental group iv, whereas in experimental groups ii and iii, 50% mortality was reached on day 7 (95% CI: 5-8) in Table 2. All small weight-class ticks in these three experimental group had died by day 10 ($P < 0.001$) as shown in Fig. 2A. Survival of ticks inversely correlated with the increasing time of submersion.

Ticks in the medium weight class (10-30 mg): Individuals in experimental groups iii, iv and vi had significantly higher mortality than did those in negative control (group i) and DW-float (v) groups ($P < 0.001$) as in Fig. 2B. Group iii vs i and group iv vs i were associated with significant mortality ($P < 0.05$). Survival of group ii vs i was observed ($P > 0.05$). Group v had no test possible because ticks were not dead.

Ticks in the large weight class (>70 mg): No tick in this weight class died after any of the experimental treatments as in Table 2 and Fig. 2C.

The effects of water or drugs on egg production: The mean egg weight for each group of ticks is presented in Table 3. Small ticks did not lay eggs because they were immature. For ticks in the medium weight class, egg weights did not differ significantly among groups i-v (Table 3 and Fig. 3). Most ticks in the amitraz solution (group vi) died and none laid any eggs. Egg weights in the largest weight class similarly did not differ significantly among groups i-v (Table 3 and Fig. 4), and a few ticks in the positive control group (group vi) laid a few eggs.

Stigmata and body surface areas in relation to water entry: Areas of the openings of stigmata were measured for each tick, as were body surface areas. In the smallest weight class, the openings of stigmata comprised 1.066% of the body surface area (Table 4 and Fig. 5A). For medium and large weight classes, corresponding values were 0.257% and 0.089% (Table 4 and Fig. 5B, C). Openings of stigmata were therefore much larger relative to body surface area in the smallest weight class, suggesting a possible route for the entry of water.

Mechanism of water entry into the body of ticks: PI solution was found inside small ticks, indicating that water had entered the body. The fluorescence intensity in ticks of this weight class was high when compared with ticks in both of the larger weight classes (Fig. 6A,B). PI stained the stigmata and tracheal trunks in small ticks (Fig. 6C), but this was not seen in larger weight classes (Fig. 6D). However, ticks of all weight were positive for fluorescence in the gut, including the salivary glands.

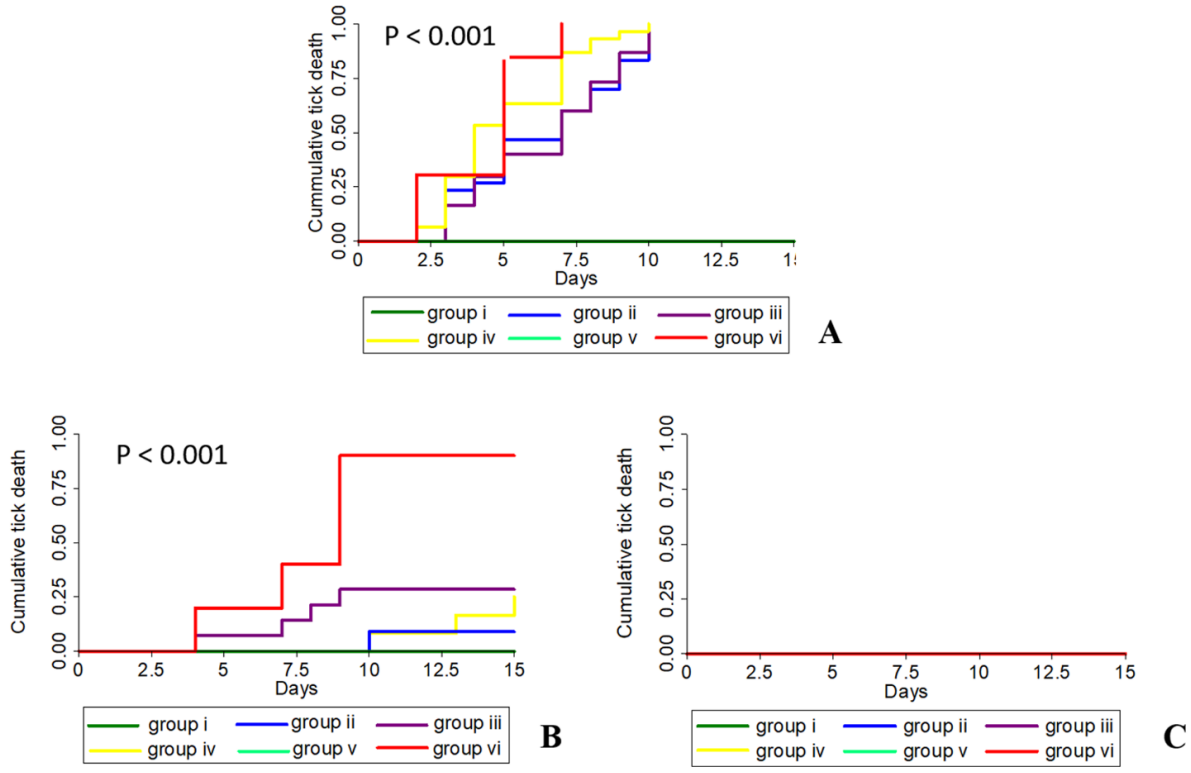

Figure 2 Cumulative tick death in the tick weight class. A value of P < 0.05 was considered as statistically significant. (A) overall of small weight class, (B) overall of medium weight class, (C) overall of large weight class.

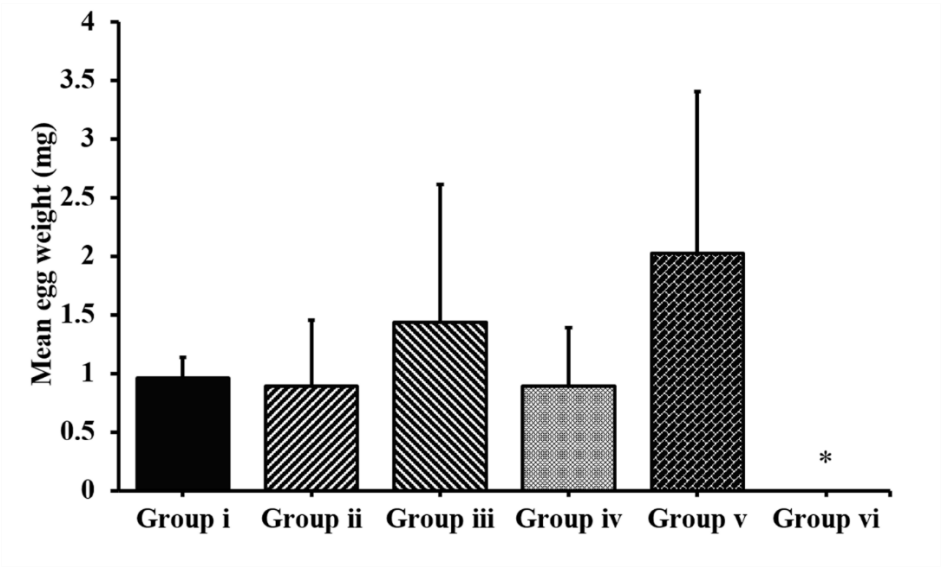

Figure 3 Mean egg weights of cattle ticks in the medium weight class (10-30 mg) analyzed using non-parametric Kruskal–Wallis test and Mann–Whitney *U* test* .Statistically significant compared with the normal control group ($P < 0.05$). Pairwise comparisons between groups other than the positive control were not significant.

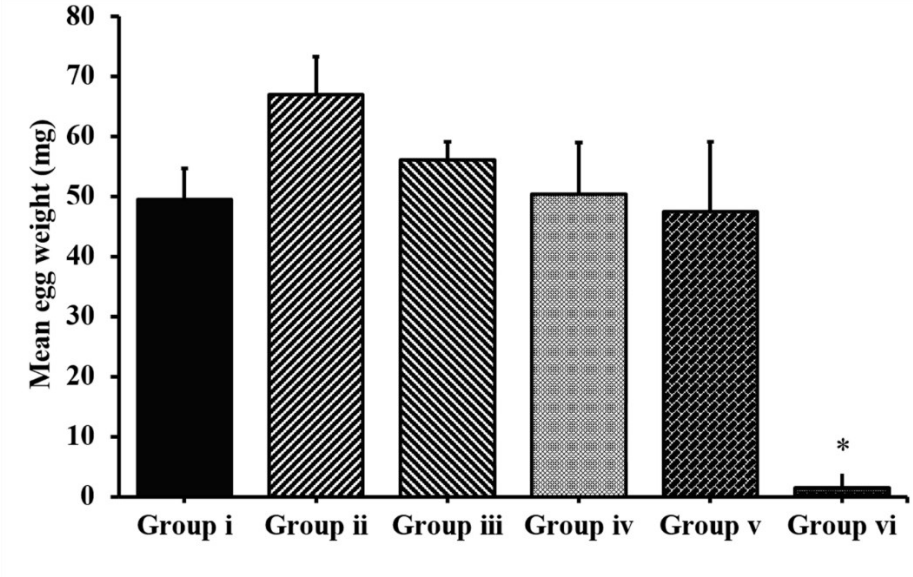

Figure 4 Mean egg weight of cattle ticks in the largest weight class analyzed using non-parametric Kruskal–Wallis test and Mann–Whitney *U* test,* Statistically significant compared with the control ($P < 0.05$). Pairwise comparisons between groups other than the positive control were not significant.

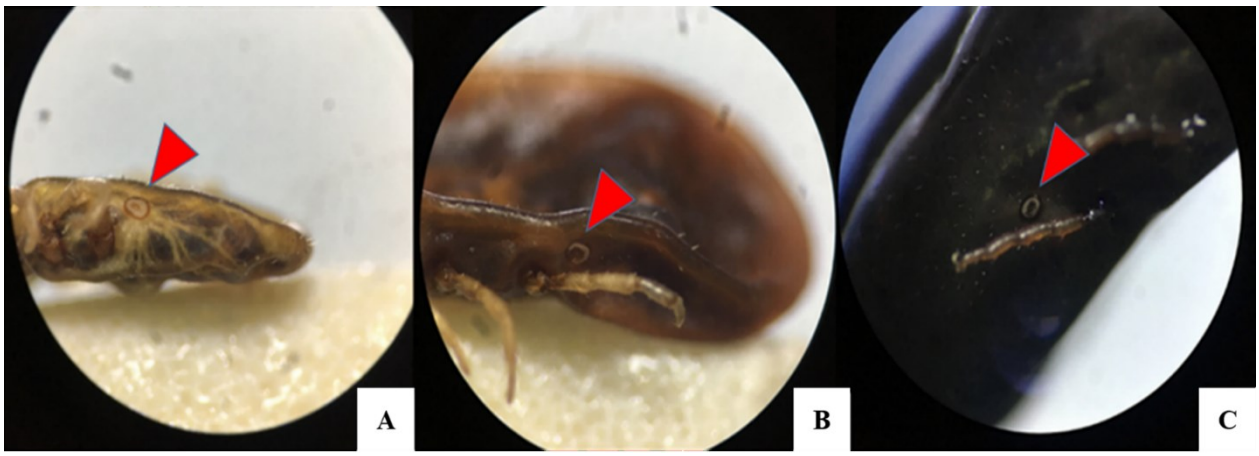

Figure 5 Stigmata of cattle ticks. (A) 3-10 mg tick, (B) 10-30 mg tick, (C) over 70 mg tick, the arrow indicates site of stigmata

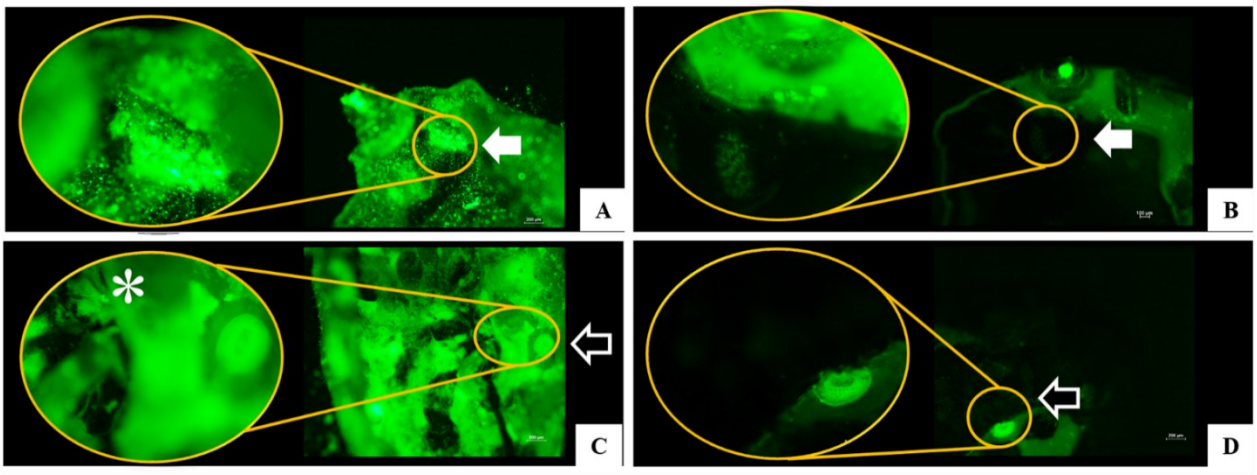

Figure 6 The ticks were submerged in propidium iodide solution for 15 mins then cut on horizontal longitudinal plane and photographed. (A) dorsal half of small size tick; (B) dorsal half of large ticks; (C) ventral half of small ticks; (D) ventra1 half of large ticks. Empty arrow indicates salivary glands. Solid arrow indicates stigma. Asterisks indicate tracheal trunks.

Table 2 Median overall survival by group (95%CI)

Size class	Small		Medium		Large	
Treatment	**Median survival (95%CI)**	***P*-value[a]**	**Median survival (95%CI)**	***P*-value[a]**	**Median survival (95%CI)**	***P*-value[a]**
i normal control	-	-	-	-	-	-
ii 5 mins DW	7 (5-8)	*P* < 0.001 (Gr. ii vs Gr. i) *P* = 0.007 (Gr. ii vs Gr. vi)	-	*P* = 0.243 (Gr. ii vs Gr. i) *P* < 0.001 (Gr. ii vs Gr. vi)	-	NA (Gr. ii vs Gr. i) NA (Gr. ii vs Gr. vi)
iii 10 mins DW	7 (5-8)	*P* < 0.001 (Gr. iii vs Gr. i) *P* = 0.005 (Gr. iii vs Gr. vi)	-	*P* = 0.043 (Gr. iii vs Gr. i) *P* < 0.001 (Gr. iii vs Gr. vi)	-	NA (Gr. iii vs Gr. i) NA (Gr. iii vs Gr. vi)
iv 15 mins DW	4 (4-7)	*P* < 0.001 (Gr. iv vs Gr. i) *P* = 0.358 (Gr. iv vs Gr. vi)	-	*P* = 0.028 (Gr. iv vs Gr. i) *P* = 0.008 (Gr. iv vs Gr. vi)	-	NA (Gr. iv vs Gr. i) NA (Gr. iv vs Gr. vi)
v 15 mins DW (float)	-	NA (Gr. v vs Gr. i) *P* < 0.001 (Gr. v vs Gr. vi)	-	NA (Gr. v vs Gr. i) *P* < 0.001 (Gr. v vs Gr. vi)	-	NA (Gr. v vs Gr. i) NA (Gr. v vs Gr. vi)
vi 5 mins amitraz	5 (2-5)	*P* < 0.001 (Gr. vi vs Gr. i)	9 (4-9)	*P* < 0.001 (Gr. vi vs Gr. i)	-	NA (Gr. vi vs Gr. i)

Abbreviation: CI, confidence interval; Gr, group; NA, no test possible because ticks were not dead.
[a] p-value computed from log-rank test comparing treatment vs control group (normal control and positive control)

Table 3 Mean weight (± SD) of eggs produced by adult female *Rhipicephalus* (*Boophilus*) *microplus* in each group

Size class Treatment	Small (mg)	Medium (mg)	Large (mg)
i normal control	0	0.96±0.18	49.43±5.25
ii 5 mins DW	0	0.89±0.57	66.90±6.34
iii 10 mins DW	0	1.43±1.18	56.04±3.05
iv 15 mins DW	0	0.89±0.50	50.29±8.70
v 15 mins DW (float)	0	2.02±1.38	47.35±11.72
vi 5 mins amitraz	0	0	1.34±3.00

Table 4 Stigmata area, body area and ratio of stigmata area and body area of adult female *Rhipicephalus* (*Boophilus*) *microplus* in each size

Size class Structure	Small	Medium	Large
Stigmata area (mm^2)	0.104±0.005	0.088±0.003	0.059±0.004
Body area of ticks (mm^3)	10.170±2.095	35.117±5.676	68.085±13.387
Ratio of stigmata area and body area (%)	1.066	0.257	0.089

Discussion

We demonstrated that water immersion affects the survival of ticks correlated with the weight of tick and length of immersion. In ticks of the lowest weight, openings of the stigmata were proportionally much larger than in heavier ticks. This might provide a route of entry for water and explain why small ticks were so sensitive to immersion.

Rhipicephalus microplus is one host tick, which can found at all stages in one host. Previous studies have looked at acaricidal activity including adult immersion test, larval packet test and larval immersion test (Klafke *et al*., 2006; Higa *et al*., 2016; Sugauara *et al*., 2019). All tests are recommended by The Food and Agriculture Organization (FAO, 2004). Many previous studies used both adult and larval stages for acaricidal activity and the lethal concentration that killed adult female ticks was higher than the larval stage (Zaman *et al*., 2012; Shyma *et al*., 2014; Sugauara *et al*., 2019). This could imply that the larval stage is more affected than adult females. Therefore, the adult immersion test is the most popular acaricide test which supports our present study. However, our preliminary investigation found that the male tick died earlier (median survival at day 6) than the female tick (median survival at day 7) after the water immersion test.

Chemical control by acaricides is becoming ineffective. Ticks are acquiring resistance. There is frequent inappropriate use of acaricides and lack of knowledge on strategies for delaying acaricide resistance among farmers (Sungirai *et al*., 2016; Bandara and Karunaratne, 2017). There are several biological tick controls. Predation on ticks by chickens can be used as a control method in a poor urban environment (Hassan *et al*., 1991). Modification of habitats is vegetative management (Mount, 1981). Herb acaricides can be used against ticks. These are cheap, especially for farmers in developing countries (Godara *et al*., 2018). Furthermore, some herbal products have dermatitis, psychological and neurological side effects. Alternative methods of controlling ticks are needed. Our study was prompted by the observation that buffaloes, spend much of their time immersed in water and are rarely infested with ticks.

Small ticks absorb water more readily than do large ticks. Openings in the body through which water can pass include the mouth and the stigmata. Ticks of all sizes were positive for fluorescence in the salivary glands, suggesting some entry of water through the mouth. This was clearly not sufficient to injure larger ticks. Kim *et al*., (2017) showed that tick salivary glands function with water balance. Water entering through the stigmata may explain the intensity of fluorescence in the tracheal trunk of small ticks but this organ does not fluoresce in large ticks.

Previous studies support our present study that ticks underwater can survive, including unfed ticks and engorged ticks. They found that unfed ticks can survive after submerging in water for 24 days (Bidder *et al*., 2019) and engorged female ticks can survive in water immersion for 48 h (Giannelli *et al*., 2012). The possible mechanism for survival underwater has been reported by Fielden *et al*., (2010); the spiracle function as a plastron that can absorb oxygen from water via a thin layer of air trapped by hydrophobic hairs or other cuticular projections. There is spiracle plate with numerous pores surrounding the ostium. These pores are aerophytes and open into internal air spaces. Gas exchange with ambient air takes place. After ostium cross-section, the atrium opens into the tracheal trunks. But the ostium is not thought to participate in gas exchange in the adult tick. Our results found that the largest weighing ticks have a small stigmata size. Therefore, little water enters into the air chamber and the engorged female tick can survive. Our study agrees with the previous study that engorged female ticks survived water immersion. In contrast, our new finding is that feeding ticks cannot survive post water immersion. The reason for feeding ticks not being able to survive may from the water easily passing through the stigmata pore as shown in figure 5, and being weak during early feeding because of the need more blood for the synthesis of energy, yolk protein and vitellogenins (Kopáček *et al*., 2018).

In conclusion, the simple technique of immersion in water can be used to kill cattle ticks. Length of immersion and weight of tick are important variables influencing the outcome. Immersion for at least 5 mins can kill ticks, especially those of the small size of female ticks. Immersion fails to kill large ticks, for which other methods must be employed. However, the routine killing of small ticks by immersion will limit population growth overall.

This present study indicates a basic knowledge of water immersion is a useful, simple, safe and cheap technique. It can be applied to control ticks in production animals and/or pets by walking or soaking in a water tank or swimming in a water reservoir without using any chemical reagents.

Acknowledgements

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