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Article

The underwater survival of adult and larval stages of *Argas persicus* (Acari: Argasidae)

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Abstract

The soft tick, *Argas persicus*, is the most important ectoparasite of chicken and other domestic fowl, as well as humans. The main aim of this study was to evaluate the survival rate of *A. persicus* under three different environmental conditions, including immersion in tap water, immersion in oxygenated water and after being placed and washed along clothes in an ordinary washing machine. Adult and larval ticks were separately immersed in tap and oxygenated water for six different time periods: 1, 6, 24, 48, 72 hours and 1 week. Both adults and larvae survived up to 72 hours immersion in tap water. All the ticks immersed in tap water died during the first week; while 53.3% of adult ticks survived one week immersion in oxygenated water. To evaluate the survival rate of ticks in the washing machine, only adults were used and the ticks were placed in a small hub filter before being placed in a Samsung washing machine at three different temperatures (30, 40 and 60 °C). At the end of each cycle, we counted the survived ticks. All ticks survived 30 and 40 °C, while only 13.3% survived at 60 °C. In conclusion, adult and larval stages of *A. persicus* survived under water for couple of days while washing in regular washing machine did not kill adult ticks under temperatures of 30 and 40 °C. So, in order to kill the ticks in clothes temperatures more than 60°C should be used.

Key words: *Argas persicus*; chicken, domestic fowl; ectoparasite; tick; survival rate.

Introduction

The soft tick, *Argas persicus*, is the most important ectoparasite in poultry, which was first recorded by Lorenz Oken in 1818 in Mianeh, Iran (Fortescue 1924). Despite its high prevalence in different parts of Iran, it has also a worldwide distribution in warm climate. Apart from causing anaemia, anorexia, weight loss and depressed egg output, *A. persicus* is the main vector of *Borrelia anserina* (the causative agent of the avian spirochaetosis) and *Aegyptianella pullorum* (Leeflang and Ilemobade 1977). It is also capable to transmit *Mycobacerium avium, Pasturella multocida*, West Nile virus, *Salmonella gallinarum*, *S. pullorum, Mycoplasma gallisepticum and M. meleagridis* to poultry (Stefanov *et al*. 1975; Hoogstraal 1985; Soliman *et al*. 1988). Tick paralysis in chickens, a flaccid motor paralysis, may result from attacks by larvae of *A. persicus* (Rosenstein 1976). In addition to chickens and other domestic fowl, it also feeds on humans, although immunity has been acquired by some individuals (Trager 1940). Accordingly, the control of human infection with this tick would greatly reduce the incidence of diseases associated with the pathogens transmitted by *A. persicus*.

A waterproof integument inhibits water loss and helps ticks to tolerate prolonged off-host existence. In nature, the ability of off-host parasitic stages to endure immersion is important for the survival of ticks after heavy rainfall events or temporary flooding. This may be due to an alternative respiratory system, by means of using spiracular plates that allow them to absorb oxygen from water (Fielden *et al*. 2011). A tick may spend up to 24 hours on the host before biting and then feed from hosts' bodies from 2 hours to 7 days before dropping off (Hoogstraal 1985). Most people, who become accidentally infested by ticks, can carry them with clothes to their home (Carroll and Kramer 2001). If tick infested clothing is removed and left somewhere, the risk of tick bite still exists.

Apart from studies that examined the sensitivity of hard ticks to laundering and under water survival (Barrett *et al.* 2009; Giannelli *et al.* 2012), there are not enough studies on underwater survival of soft ticks and their survival to laundering. The aim of this study was to examine the survival of *A. persicus* under water and its resistance to typical laundering practices.

Material and methods

The *A. persicus* ticks are active during the night. They spend the daytime hidden in cracks and crevices of the walls of chicken houses or wooden materials such as windows or doors of poultry-roosting areas. Therefore, these places were observed for the presence of ticks in some villages of Urmia City, West Azerbaijan Province, Iran. Special attention was paid to presence of tick faeces, as tick habitats are marked by accumulation of excrements. At each infested site, several specimens at different developmental stages were found, and then transferred to the Department of Parasitology, Faculty of Veterinary Medicine of Urmia University where their developmental stages and sex were determined using morphological characteristics (Wall and Shearer 1997). Consequently, the ticks were transferred into Petri dishes and kept at 28 °C with 80% relative humidity (RH) in an incubator. Afterwards adult ticks were used for experiments.

Some engorged adult female ticks were kept in desiccators at room temperature (22–25 °C) with 80% RH for egg deposition. The two-week rested eggs were collected and kept in desiccators at 25 °C to obtain tick larvae. Afterwards the obtained larvae were placed in a separate sterile vial. In order to evaluate the underwater survival of an adult *A. persicus* tick, 18 test tubes were filled with tape water. Five ticks were placed in each tube. Test tubes were kept in the laboratory conditions for 1, 6, 24, 48, 72 hours and one week. Three replicates were considered for each time period. At each time interval the water was discarded and ticks were dried with Whatman filter paper. Ticks were observed for survival separately. The tick showing the slightest lethargic and sedentary movement was considered alive.

The same procedure was followed for *A. persicus* larvae. Fifteen day old tick larvae were left immersed in water filled microtubes for 1, 6, 24, 48, 72 hours and one week.

Adult ticks were also examined for their survival in oxygenated water. For this purpose, oxygen was pumped into the water using a ps950 pump. Five adult ticks were placed in a small hub filter which was placed in a container filled with water and kept for periods of 1, 6, 24, 48, 72 hours and one week. Three replicates of each interval were included in the experiment. After each time interval the water was discarded and ticks were investigated for survival.

To investigate survival rate of ticks in the washing machine, five adult ticks were placed in a small hub filter inside the clothes, were loaded in a b1280d Samsung washing machine with one cup of washing machine powder (Top loader washing machine powder, Paknam Co.) as per common washing method in an ordinary household. The experiment was carried out using three programs at 30, 40 and 60 °C temperatures for 35 min. Three replicates were considered for each temperature. At the end of each washing time, hub filters were taken out of washing machine and the ticks were dried with Whatman filter paper and checked for survival.

Statistical analysis of data was performed using one way ANOVA, using Statistical Package for the Social Sciences (SPSS, Version 21, Chicago). Values of $P < 0.05$ were considered as a significant difference between study groups.

Results

Ticks survivals in tap water and oxygenated water are shown in Table 1. In each trial, the survival rate decreased as immersion time increased. Survival rate for tick in tap water and oxygenated water were significantly different. All 15 immersed adult ticks in tap water survived for 6 hours, 8, 5 and 3 out of 15 ticks were alive after 24, 48 and 72 hours, respectively. No tick survived after 1 week ($p < 0.05$).

All 15 larvae survived for the 1 hour immersion, 12 larvae for 6 and 24 hours, 10 larvae for 48 hours, 8 larvae for 72, but all 15 died during 1 week ($p < 0.05$). The ticks that were immersed in the oxygenated water had higher survival rate than the tap water. All ticks were alive in periods of 1, 6, 24 and 48 hours. Eleven ticks in 72 hours and 8 ticks in 1 week were alive ($p < 0.05$). Ticks immersed in oxygenated water had a very short recovery time in comparison to the tap water immersed ticks.

The results for the ticks that were placed in the washing machine indicated that all ticks were alive when the machine was programmed at 30 and 40 °C and just only two survived at 60 °C, then died in less than one hour ($p < 0.05$).

Table 1. Ticks survivals in tap water and oxygenated water

	Ticks survival rate (%)					
Treatment (n = 15)	1h	6h	24h	48h	72h	1w
without oxygenation (adults)	100^{a}	100^{b}	53.33^{abc}	33.33^{ab}	20^{ab}	0^{abc}
without oxygenation (larvae)	100^{h}	80^{i}	80^{j}	10^{k}	53.33	0^{hijk}
with oxygenation (adults)	100^{d}	100^{e}	100^{f}	100^{g}	73.33	53.33^{defg}

* Similar letters in each row indicate significant differences between the groups

Discussion

This is the first study that shows adults and larvae of *A. persicus* can survive immersed in water for a specific period of time, especially adult ticks that are immersed in the oxygenated water for up to seven days. Adult ticks and larvae survived in tap water for 72 hours, while there was no resistance to immersion for one week. However, Smith (1973) reported a 9 to 14 days average survival time for *Amblyomma variegatum* (Fabricius) which shows that hard ticks are more resistant to immersion than soft ticks. In contrast, in the study performed by Giannelli *et al.* (2012), all *Rhipicephalus sanguineus* (Latreille) female ticks were reported to have survived a 48 hours immersion and some survived up to 72 hours immersion, but none of them survived after a longer period (Giannelli *et al.* 2012).

Recovery time after immersion varied from a few minutes to several hours. Long recovery was associated with prolonged immersion time. Recovery time for ticks immersed in oxygenated water was much shorter than in the tap water.

The reduction of survival time in tap water compared with oxygenated water revealed the importance of dissolved oxygen in submerged ticks as a means to sustain aerobic respiration. Thus the oxygen obtained from the water is of great significance. Interesting results were obtained from a study on American dog tick, *Dermacentor variabilis* (Say), immersion in hypoxic and normoxic water. Ticks immersed in hypoxic water and normoxic water had survived up to 9 days and 14 days, respectively (Fielden *et al.* 2011).

The resistance to water immersion has also been linked to the use of a spiracular system in certain invertebrates that allows oxygen dissolved in water to diffuse into the tracheal system according to its concentration gradient (Fielden *et al.* 2011). The spiracular plate in ticks differs markedly from plastrons described for most other arthropods. In adult insects, the plastron typically involves a dense array of hydrofuge hairs on the cuticle (Hinton 1971).

In mites, the peritreme of the spiracle is lengthened to form an open groove in the cuticle of the body wall with small microscopic hydrophobic cuticular hairs projecting from the bottom that trap a layer of air (Hinton 1971; Krantz 1974). In Amblypygi which are non-tracheate, cuticular projections around the openings of the book lungs are able to retain a layer of gas (Hebets and Chapman 2000).

Some species of insects which are subjected to hypoxia as a result of submergence in water are capable of decreasing their metabolic rates and of switching to anaerobic metabolic pathways (Hoback and Stanley 2001; Zerm *et al.* 2004). In a study done by Carroll and Kramer (2001), after washing ticks in cold and hot water for 30 minutes, almost all of (90%) *Amblyomma americanum* (Linnaeus) and *Ixodes scapularis* (Say) nymphs survived. All nymphs of both species were dead when the high heat drying cycle was used for an hour. However, in our study ticks survived at 30 and 40 °C whereas only 2 ticks were alive at 60 °C.

It is therefore concluded that both adults and larvae of *A. persicus* survived under water for a couple of days. Washing in regular washing machine did not kill adult ticks under temperatures of 30 and 40 °C. So in order to kill the ticks in clothes, temperatures more than 60°C should be used. Given the laundering recommendations of clothing manufacturers and variations in the use of automatic clothes washers, laundry washed in automatic washers should not be considered free of living ticks.

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زنده‌مانی نوزاد و کنهٔ بالغ *Argas persicus* (Acari: Argasidae) در زیر آب

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چکیده

کنهٔ نرم *Argas persicus* انگل خارجی با اهمیت ماکیان و سایر طیور اهلی و انسان است. هدف اصلی مطالعهٔ حاضر بررسی زنده‌مانی *A. persicus* در سه وضعیت فرو بردن در آب معمولی، آب اکسیژن‌دار و شستشو به همراه لباس‌ها در ماشین لباسشویی است. کنه‌های نوزاد و بالغ در درون آب معمولی و آب اکسیژن‌دار در شش زمان گوناگون شامل ۱، ٦، ٢٤، ٤٨، ۷۲ ساعت و یک هفته نگهداری شدند. تمام نوزادان و بالغ‌ها پس از ۷۲ ساعت در آب زنده ماندند. تمام کنه‌ها در آب معمولی از بین رفتند در حالی که ۵۳/۳٪ کنه‌های بالغ در آب اکسیژن دار زنده ماندند. به منظور بررسی زنده ماندن کنه‌ها در ماشین لباسشویی تنها از کنه‌های بالغ استفاده شد. کنه‌ها را در ظرف سیمی کوچک گذاشته و در سه برنامه دمایی (۳۰، ٤٠ و ٦٠ درجهٔ سلسیوس) در ماشین لباسشویی مدل سامسونگ قرار داده شدند. پس از پایان هر برنامه شستشو کنه‌های زنده شمارش شدند. تمام کنه‌ها در دمای ۳۰ و ٤٠ درجهٔ سلسیوس زنده ماندند در حالی‌که ۱۳/۳٪ آنها در ٦٠ درجهٔ سلسیوس زنده بودند. با توجه به نتایج به دست آمده کنه‌های *A. persicus* در مرحلهٔ لاروی و بالغ به مدت چند روز در زیر آب زنده می‌مانند هم چنین شستشو در دمای ۳۰ و ٤٠ درجهٔ سلسیوس در ماشین لباسشویی نمی‌تواند کنه‌های بالغ را از بین ببرد. بنابراین به منظور از بین بردن کنه‌ها باید لباس‌ها در دمای بیشتر از ٦٠ درجهٔ سلسیوس شسته شوند.

واژگان کلیدی: *Argas persicus* ، ماکیان، طیور خانگی، انگل خارجی، کنه، میزان زنده‌مانی.

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