

Theileriosis in Grazing Sheep and its Interrelation with the Reptiles Ticks

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Abstract: The main aim of this study was to found out about complaints made by herdsman and one of the main sources of Theileriosis in sheep's go out for grazing early morning and back in lat evening daily in Zakrous mountain and forest area, western of Iran close border with Iraq. A total of 568 blood samples from 23 herds of sheep were examined for the presence of *Theileria* spp. before experimental study all the animals their fence and area were sprayed by insecticide to conform the animal are free from any external parasites. 12 animals as control from same herds were kept separately without sending them out for grazing. Study of 129 reptile (*Trachylepis vittata*, *Trachylepis a. transcaucasica*, *Trapelus lessonae* and *Laudakia nupta*) specimens was collected during May 2008 to August 2011. The prevalence of *Theileria* spp was observed in ring, oval and rod forms in the rats of 25.35% in sheep. The frequency of the ticks on the body of the sheep's were *Hyalomma* spp (46.88%) - *Repicephalus* spp (42.32 %) and -.0. *Haemaphysalis* spp (10.78%). Out of 129 lizards species 78 (60.46%) cases had infestation with ticks (*Hyalomma aegyptium*, *Haemaphysalis* spp and *Repicephalus sanguineus*). Prevalence of *Hyalomma aegyptium*, *Repicephalus sanguineus* and *Haemaphysalis* spp were 39.53%, 13.95% and 17.05% respectively. *Hyalomma aegyptium* was found to be the most frequent tick species. In this study, 62 of specified ticks were male and 67 were female. Prevalence of ticks in male lizards was 61.29% and in female one was 53.73%. Out of 25 turtle, one of them was infested only by one tick. *Hyalomma aegyptium* were recognized on and attachment site were on the front leg of the *Testudo graeca* turtle. Conclusion: The combination of ticks found in this study shows there is some relationship for transmit ion of the theileriosis from reptiles to grazing sheep and this phenomena is permanent or accidental relationship is not clear. Observation shows that when the grazing sheep take rest during the day in the area were the reptile are living, at this time ticks transfer to the animals. It can be concluded that if reptiles represents any ticks, it will be limited on their territory.

Key word: Ticks • Ruminant • Lizard • Tourtile

INTRODUCTION

The main aim of this study was to found out the reasons' behind complain made by herdsman using different chemical insecticide and again facing theileriosis in their herds and searches for one of the main sources of Theileriosis in sheep's go's out for grazing early morning and back in lat evening daily in Zakrous mountain and forest area, western of Iran close border with Iraq. The primary field investigation shows that reptiles in the area were the sheep go daily for grazing is infested by ticks.

Ticks are one of the most significant parasites of animals and man globally, particularly in tropical and subtropical regions [1]. Approximately 10% of 899 tick

species take role in transmission of more than 200 pathogens [1, 2], including many zoonotic agents: bacteria, rickettsia, protozoan and virus [1, 3]. The transfer of ticks on hosts outside their natural territorial habitats is facilitated and maintain by the animals seasonal journeys and migrations, by the development of means of transport, human tourist travel and also by human activity related, among other things, to the trade in animals [4].

Parasites of *Theileria* spp. infect wild and domestic animals in the tropical and subtropical regions of the world. Ruminants maintain a valuable economic and ecological niche in Asian agriculture [5]. Majority of the livestock owners earn their livelihood through sale of surplus animals and their by-products. There are several

factors affecting the production potential of livestock. Among these, parasitic infestations are widespread, affecting different livestock species throughout the world. These cause considerable economic losses in terms of low productivity and mortality in small ruminants [6]. The only common feature between these diseases is that they can all be transmitted by ticks. Theileriosis and Babesiosis belong to this complex and cause diseases in the livestock with high morbidity and mortality and therefore they give rise to the high economical losses worldwide [7, 8]. *Theileria parva* and *T. annulata* cause serious problems for the health and management of the cattle in tropical and subtropical regions [9]. More recently, interest has been focused on the sheep-infecting *Theileria* parasites. Among known *Theileria* parasites of sheep, *Theileria lestoquardi* and *Theileria* spp. from North China are considered highly pathogenic. The other species, *Theileria ovis*, *Theileria separata* and *Theileria recondita* cause subclinical infection in small ruminants [10].

Among these, *Theileria lestoquardi* causes malignant theileriosis in sheep and goats, a severe lymphoproliferative disease with high mortality and morbidity [11, 12]. *T. lestoquardi* is transmitted by ticks belonging to the family *Ixodidae* causing fever, lymphadenopathy, wasting, anemia and jaundice [13].

The international trade in reptiles has grown dramatically in the last decade, with the United States responsible for more than 80% of the total world trade in live reptiles listed by the Convention on International Trade Endangered Species of Wild Fauna and Flora [14, 15]. It has been known for many years that reptiles imported into the United States often were infested with ticks [16] and, recently, it was shown that exotic ticks introduced on imported reptiles could be infected with organisms pathogenic to domestic livestock populations [17].

According to previous survey Iran has 13 geographical regions relative to lizard distribution [18, 19]. The geography of Ilam province includes three of these regions, the Zagros Mountains, the Khuzestan plain and the western foothills of the Zagros Mountains. Two climatic conditions, Mediterranean and Dry Semidry, exist in Ilam province. More than 78% of the province is covered with forests meadows and arid lands, thus the area should have a rich biodiversity of lizards in general and of ticks in particular.

The genus *Hyalomma* distribute in Africa, southern Europa and Asia [20]. The first report of presence of *Hyalomma aegyptium* tick from *Testudo graeca* turtle in

Iran were reported by Nabian and Mirsalimi [21]. There is no any report regarding external parasites of lizard from Iran. The tortoise tick *Hyalomma aegyptium* is among the easily recognizable species. It possesses a set of morphological characters for instance two equal, well separated spurs of coxa I in both sexes. A number of other genera of tick also parasites reptiles: *Argas transversus*, *Bunopus tuberculatus* and *Cryptopodion heterocercum*. This study is aiming to investigate reptile ticks from Ilam, Western Province of Iran as the first report in region.

The present investigation was planned to find out the prevalence of ticks and theileriosis in tick infested sheep and reptiles maintained in Ilam province, located in western of Iran.

MATERIALS AND METHODS

Area Study: Western Iran in general and Ilam province in particular, Zakrous mountain and forest area has unique geographical and climatic condition that supports a rich flora and fauna. Ilam province is located in western of Iran in the vicinity of Iraq country. The length of common Iran-Iraq borderline is 465 Km.

Animal Sampling: Study on herds 23 combination of minimum from 42 to 86 sheep daily early morning go out for grazing in Zakros mountains area and back at lat evening, blood sampling totally of 568 randomly selected sheep's from mention herds were collected, before experimental study all the animals their fence and area were sprayed by certain insecticide to conform the animal are free from any external parasites. 12 animals as control from same herd's were kept separately without sending them out for grazing; feed and water at leptom under control, all the animal once week were checked for ectoparasites.

Temperature (°C) was monthly recorded. For collection of ectoparasites special tray white slippery with opening hole in the middle and jar class were fixed on this hole, animals were moved and held over this white tray. For detecting or collecting ectoparasite the bodies of animals were combed and their skin rubbed with a piece of cotton sucked in ether to remove the ectoparasites. In the case of collecting mites we had search does part of the sheep body how suffering from hair or wool loss, alopecia or swelling with the help of scalpel contaminated with oil or glycerin, the ectoparasites were separated and baggies in 75 % ethanol. All ectoparasites were counted at 40× and identified at 400×microscopically. The sample were proceed in lactophenol, Hoier or xialine and for

conforming identification slide- mounted in Canada balsam and then examine under a binocular or high power microscopy until they were identified (male or female and species) according to the Center of Disease control (CDC) key. Each slide was completely and carefully examined microscopically. The wool was initially clipped and then a superficial skin scraping was performed. The specimen was mounted on a glass slide with mineral oil preparation.

The Statistical Analyses System (SAS, Cory, NC, USA) was used for all analyses. All data were tested for Gaussian distribution and submitted to one-way ANOVA.

The Local Ethical Committee in Veterinary Researches as well as the Research Department in Ilam University approved the proposal of the present study.

Tick Collection from Reptiles: The area where the herds of animals were go daily for grazing, with the help of net any kind of reptile were seen, caught and checked for ectoparasites.

A total of 129 lizard 25 tortoise specimens were collected during May 2008 to August 2011. Ticks were collected from reptilian using tweezers, immediately put into plastic tubes containing 70% ethanol and labeled with a field number of the reptilian specimen. Ticks were identified according to Aydn [22].

Daily routine movement of reptiles was measured by two different ways, to find out about transmissions of ticks to other host, for this reason reptiles were free in open surrounded circle with diameter of 100× 100 meter area carpet with the soft fine wood powder (fine saw dust) for 24 hours and the sign remain in the ground at the end of the day were measured, each lizard were released and freed separately in this area and distance of their movement were measured. In other method the lizard were marked with color in their back and released in natural field and then followed by distance with string in the ground then at the end of the day the string were measured to obtain their distance of movement per day.

RESULTS AND DISCUSSION

A total of 568 blood samples from sheep were examined for the presence of *Theileria* spp. Blood smears were made and stained with Giemsa and examined under light microscopy for the presence of piroplasms in the erythrocytes of the infected animals. The prevalence of *Theileria* spp. was found to be 12.85% (n=73) in sheep. The *Theileria* spp was observed in ring, oval and rod forms, with a size of 0.5–1.5 mm. The parasitemia rate ranged from 0.03 to 0.2% in the infected animals.

The prevalence of *Theileria* infection was found to be 10.6% and 14.05% in lambs and adults, respectively. The total number of infected female were 14.8% and in male 8.1%. The prevalence of *Theileria* spp. infection in all age groups and between male and female sheep was not significantly different. Durrani *et al.* [23] did not observe any effect of gender and age on the occurrence of theileria in sheep and goat. The overall prevalence rate in both sheep and goats has been found to be 11.20% in the current study.

In the present study, the prevalence of theileriosis was lower (12.85%) in sheep as compared to the study conducted by Rehman *et al.* [24] who reported 13.9% theileriosis. Razmi *et al.* [25] in the study on 840 sheep in the South Khorasan province reported 11.9% of positive *theileria* and this report in comparison to our study was significantly lower rate of infection to the theileriosis in sheep. This difference might be due to the different geographic location of the both province. Climatic conditions dictate the dynamics of tick borne diseases by affecting the distribution of ticks and their seasonal occurrence [26]. Altay *et al.* [27] reported 18.29% sheep and 2.88% goats to be infested with theileriosis in Turkey. The prevalence of *Theileria* infection was reported to be 10-36% in different areas of Iran [28-31].

In the present study, the overall prevalence of ticks was recorded as 42.4 % in sheep. Three different tick species were identified. The most frequent and abundant tick species that were found on sheep were *Hyalomma* (46.88%) and *Rhipicephalus* (42.32%) (Table1). Multiple rate of double and triple tick's infestation were found to be 11.4%, 1.3%, respectively.

The prevalence of ticks and *Theileria* was variable during different seasons of the year. Season has been found as one of the important risk factors that affects the prevalence of *Theileria* spp and ticks. Our results showed that summer season is important risk factor for the prevalence of *Theileria* spp. in grazing sheep. Prevalence of ticks was higher (43.98%) during the summer season (June-August) and the highest rate of theileriosis (50.6%) was also in the same season (Table2). The increase in prevalence during hot seasons could be attributed to tick infestation rate which is influenced by temperature, rainfall and relative humidity [32].

Out of 129 lizards species; 38, 34, 31 and 26 numbers were identified as *Trachylepis vittata*, *Trachylepis a. transcaucasica*, *Trapelus lessonae* and *Laudakia nupta* respectively. Results indicated that 78 (60.46%) cases had infestation with ticks (*Hyalomma aegyptium*, *Haemaphysalis* spp and *Rhipicephalus sanguineus*).

Table 1: The frequency of tick species collected on the body of sheep

Ticks	No. of sheep				Total
	Age		Sex		
	Lambs	Adults	Male	Female	
<i>Hyalomma spp</i>	61	52	56	57	113(46.88%)
<i>Repicephalus spp</i>	48	54	63	39	102(42.32%)
<i>Haemaphysalis spp</i>	15	11	12	14	26(10.78%)
Total	124	117	131	110	241 (42.4%)

Table 2: The compression of tick and *theileria* species collected on the body of sheep in different seasons

		No. of sheep				Overall infestation
		Age		Sex		
		Lambs	Adults	Male	Female	
Spring	Ticks	25	16	18	23	41
	Theileria	11	7	6	12	18
Summer	Ticks	68	38	57	49	106
	Theileria	12	25	16	21	37
Autumn	Ticks	34	43	39	38	77
	Theileria	4	11	9	6	15
Winter	Ticks	9	8	6	11	17
	Theileria	0	3	2	1	3

Table 3: Prevalence of ticks in lizards

Ectoparasite	<i>Trachylepis vittata</i>		<i>Trachylepis A. transcaucasica</i>		<i>Trapelus lessonae</i>		<i>Laudakia nupta</i>		Overall percentage
	M	F	M	F	M	F	M	F	
<i>Hyalomma spp</i>	5(31.2%)	7(31.8%)	3(16.6%)	2(12.5%)	8(47.05%)	5(35.71%)	6(54.5%)	2(13.1%)	38(29.4%)
<i>Haemaphysalis spp</i>	3(18.7%)	4(18.1%)	1(5.5%)	4(25%)	2(11.7%)	0	5(45.4%)	3(20%)	22(17.05%)
<i>Repicephalus sanguineus</i>	2(12.5%)	0	3(16.6%)	1(6.25%)	3(17.6%)	4(28.5%)	1(9.09%)	4(26.6%)	18(13.9%)
Total parasites	10(62.5%)	11(50%)	7(38.8%)	7(43.7%)	13(76.4%)	9(64.2%)	8(72.7%)	9(60%)	78(60.4%)

Hyalomma aegyptium was found as the most frequent tick species (39.53%). Prevalence rate of *Repicephalus sanguineus* and *Haemaphysalis spp* were identified 13.95% and 17.05% respectively (Table 3). Out of 129 lizard species, 62 were recognized as male and 67 were identified female. Prevalence of ticks in male lizards was 61.29% and in female lizards was 53.73% (Table 3).

Out of 25 turtles, one of them was infested by only a tick. *Hyalomma aegyptium* were recognized on and attachment site were on the front leg of the *Testudo graeca* turtle (Pic. 1).

Rhipicephalus spp ticks nymph and adult were recovered on *Trachylepis vittata* lizard, the site of the attachment were on axillary and near front leg side of the body (Pic. 2), the same species of tick nymphs and adults were found on the top dorsal eyelid of the other species



Pic 1: Adult *Hyalomma aegyptium* front leg side of the *Testudo graeca* turtle



Pic 2: Nymphs (orange color) on the side of the body of *Trachylepis vittata* lizard



Pic 5: Adult tick on *Trachylepis a. transcaucasica*



Pic 3: Nymphs of the ticks (orange color) on the top dorsal eyelid of the *Trapelus lessonae* lizard



Pic 6: Adult and nymph tick were recognize on this species of *Laudakia nupta* lizard



Pic 4: Nymphs and adult tick on auxiliary and front leg of the *Trachylepis a. transcaucasica* lizard

of the lizard *Trapelus lessonae* (Pic. 3). Adult ticks and nymph were observed on dorsal hand of the different species of the *Trachylepis a. transcaucasica* and *Laudakia nupta* lizard (Pics. 4-6). The prevalence rates of ticks were more pronounce during June to the end of August. The infestations on lizards were more prevalent

on male than female in this area. While, the movement of different species of male and female lizards was under supervision; the results indicated that the males are more active than the female in this two species (*Trachylepis vittata* and *Trapelus lessonae*). The other result of this research on the habit of lizard movement shows that the female lizards had a speed running pause (stop) after 2.5-3 meters per 45-56 second. Male lizards had a less speed running pause (stop) after 18-24 second; it means that the male were run non stop for average distance of 5- 6 meter and than stop and started to run again. The comparison in this result is that the experimental female lizards stop and stay longer than female in the field and this make more chance for the tick to attached to the reptile. The other results of this study shows that the maximum and minimum distance of lizard movement per day were in order 365 meter and 139 meter (Table 4) mostly they stay for a long time in one place near to the bushes for hunting the insect. They move inside their own land and they do not go far from their territory. The other observation was the fighting between two lizards; this fight may be due to

Table 4: Distances movement for each lizard per a day in meter

Lizard No	Day 1	Day2	Day3	Day4	Day5	Day6
1	208	318	285	141	314	322
2	181	239	301	184	151	208
3	314	285	165	219	261	322
4	168	345	211	298	314	360
5	144	291	334	143	261	298
6	289	139	319	308	165	231
7	251	189	322	206	165	365
8	361	182	261	151	238	356
9	318	148	225	329	142	199
10	215	300	208	252	322	349
11	255	321	241	138	269	362
12	329	201	185	250	356	214

protection of territory, access to food and competition at mating time. The skins of some species of lizards were dissected and observed that the female skins are softer or less rougher and some extent thinner than the male lizards. Most of the times when we handle the lizard in the cases of the female, we found some sort of the secretion coming out of the vaginal portion possibly due to scaring reaction).

Reviewed ten species of ticks (three of the Argasidae, seven of the Ixodidae), parasitizing west palaearctic tortoises of the genus *Testudo* were reported by Barnard and Durden, 2000 and first and last presence of ticks on *Testudo graeca* turtle in Iran has been reported by Nabian and Mirsalimi, 2003. In this study *Hyalomma aegyptium* were recognized from *testudo graeca* turtle the site of tick were on front leg of turtle. In this study we found the nymph and adult tick of *Rhipicephalus spp* on *Trachylepis vittata* lizard. It shows that after hatching the eggs the larvae are crawl on their host and it is in agreement with reported that *Rhipicephalus spp* one host tick; all stages are spent on one animal. The eggs hatch in the environment and the larvae crawl up grass or other plants to find a host. The prevalence of ticks was more prevalent in June to end of August which were reported by Eisen *et al.* [33], they work on host gender and are influence by the season.

Newly attached seed ticks (larvae) are usually found on the softer skin inside the thigh, flanks and forelegs. They may also be seen on the abdomen and brisket. Our observation on the lizard in this study shows that reptiles live in their own area and they do not go far away from their territory.

Results of the present study show that the female movements of the lizard are lass and stay in one place more than male. The result also indicated that the prevalence of parasite is more on female than male. The reason could be that they stop for longer period in

field than male and there will be more chance for parasites eggs or larvae to attach to the lizard. Scali *et al.* reported that the intensity of infestation is depend on host as well as tick activity[34]. There are some publications in agreement with our result indicating that the female were more infested than the male lizard in Slovakia, Poland and Romania [35, 36]. However, Talleklint-Eisen and Eisen [37] reported opposite results supporting that male lizard were more infested than the female. Scali *et al.* reported that this phenomenon is probably related to behavioral differences and mating activity [34]. We also measured the distance that lizard move around and the data collected shows that the male are going more far than the female from their own territory but they not stay (pusses) one place than the female. Due to longer stay of female lizards, there will be more chance for expose than male lizards. Bauwens *et al.* [38] and Eisen *et al.* [33] reported that the home ranges of male individuals are larger than those of females and differ between lizard species.

CONCLUSION

As a result shows the combination of ticks found in this study their is some relationship for transmit ion of the theileriosis from reptiles to the grazing sheep and this is permanent or accidental phenomena relation is not clear, observation shows that when the grazing sheep during the day at the time of resting in the area were the reptiles are living, during this time the ticks are transfer to animals. Our observation on the lizard in this study shows that reptiles live in their own area and they do not go far away from their territory. It can be concluded that if reptiles represents any ticks, it will be limited on their own territory. However there are some concerns about tick born diseases, which could affect other animals and man, its needed further investigation.

It is highlighted here that this study is the first report of reptiles and turtle ticks from Ilam, Western Province of Iran.

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