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## Distribution of ticks (Acari: Ixodidae) infesting domestic ruminants in mountainous areas of Golestan province, Iran

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### PEER REVIEW

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#### Comments

This is an interesting and good research work in which authors evaluated prevalence, distribution, intensity and seasonal activity of ticks isolated from domestic ruminants in the highland regions of Golestan, Iran. Collected ticks were identified at the species level, counted and geo-referenced. In addition, isolated ticks were related to animal species, season and environmental data recorded during the study period. The results seem really interesting and useful for the control of ticks and tick-borne diseases in the considered area.

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### ABSTRACT

**Objective:** To determine the prevalence of ticks on cattle in the mountainous areas of Golestan province and their geographical distribution.

**Methods:** In total, 498 animals from 25 herds were selected to search for ticks in 2009–2010. Tick collection was carried out during four seasons, twice per season over a period of 12 month from March 2009 through February 2010 in two districts, Azadshahr and Ramian. Meteorological data were obtained from Iran Meteorological Organization. The geographical points recorded using a Garmin eTrex®H GPS.

**Results:** A total of 255 ticks were collected from a total of 219 ruminants including 44 sheep, 63 goats, 99 cows and 13 camels in two districts of the mountainous area of Golestan province, including Azadshahr and Ramian. Five species of ixodid ticks were identified: *Rhipicephalus sanguineus* (66.5%), *Rhipicephalus bursa* (4.6%), *Hyalomma marginatum* (19.9%), *Hyalomma anatolicum* (6%) and *Hyalomma asiaticum* (4%). The densities of infestations were calculated for sheep, goats, cows and camels 0.9, 0.79, 0.16 and 0.43 respectively. Seasonal activity of each ixodid tick infesting domestic ruminants was determined. The distribution maps showed ixodid ticks on domestic ruminants, and *Rhipicephalus sanguineus* were dominant species in the area.

**Conclusions:** Such research provides necessary information for human and animal health service managers to have a better understanding of prevention and control of vector borne diseases especially during the outbreaks.

### KEYWORDS

Hard ticks, Distribution, Domestic ruminants, Geographical information system, Iran

## 1. Introduction

Hard ticks as blood sucking ectoparasites and also vectors of human and animal diseases are considered as one of the most important arthropods. Ticks can transmit a variety of diseases such as Crimean Congo hemorrhagic fever, anaplasmosis, babesiosis, rickettsiosis, borreliosis and ehrlichiosis. Such diseases are considered as public health or veterinary problems in the communities. Anemia, dermatosis, toxemia and paralysis are other veterinary problems of ticks among livestock. They are distributed

throughout Iran, affecting animal welfare and causing significant economic losses in the country. The study of ticks was started by Delpy in Iran[1,2], after him the studies including faunistic study and possible diseases transmission to man and animal have been carried out in different parts of the country[3]. The early studies focused on the biology of ticks related to domestic or wild animal and also their relation to human health. Mazlum reported 24 species and subspecies of tick on domestic animals across the country[4]. Filippova *et al.* reported 17 ixodid species of tick on small ruminants and mammals from

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different zoogeographical zones of Iran[5], while Hoogstraal and Valdez determined 15 species and subspecies of ixodid ticks mainly from wild sheep and goats across the country[6]. In another study, Telmadarraiy *et al.* found seven ixodid ticks in West Azerbaijan province[7]. The prevalence of nine ixodid ticks on cattle was determined in Mazandaran province[8]. Rahbari *et al.* indicated 26 species and subspecies of hard ticks in Iran[3]. Recently, the occurrence of nine ixodid tick species was determined from Qazvin province[9]. These reports indicate a great tick infestation on domestic ruminants in recent years in different parts of the country.

Although there are some works on ticks in Golestan province[3,10,11], there is no information on ixodid ticks in mountainous areas of the province. The previous studies were considered as a faunistic studies with no mapping and geographical information. In the present study, authors focused on the highland area of Golestan provinces, where there is no information to display the distribution of hard ticks. Livestock infestation was surveyed to determine tick species, mapping and geographical distribution and seasonal activity of ixodid ticks infestation on domestic ruminants in 2009–2010.

## 2. Materials and methods

### 2.1. Study area

Golestan province includes an area of 20893 km<sup>2</sup> and is located in the northeast of Iran, southeast of Caspian Sea and borders Turkmenistan in the north. It has mild weather and temperate climate most of the year. Geographically, the mountainous area is located in the south of the province with coordinates of 36°50' N and 54°29' E (Figure 1).

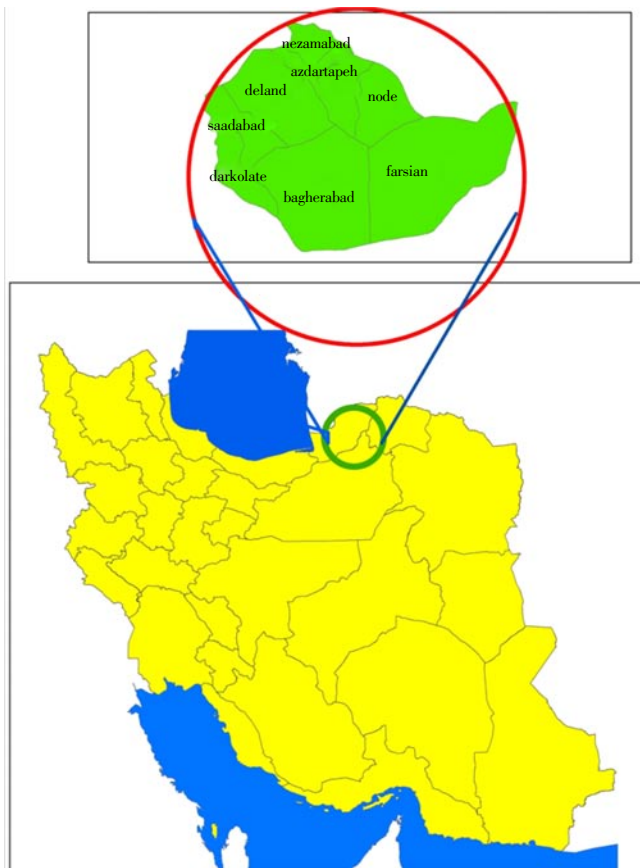


Figure 1. Map of Iran showing the mountainous area of Golestan province.

Cattle husbandry is an important source of income and employment in rural areas of the province. Sheep, cows, goats and camels are the main domestic stocks of this area.

### 2.2. Data collection

The study was conducted in the mountainous area of Golestan province in 2009–2010. Tick collection was carried out during four seasons, twice per season over a period of 12 month from March 2009 through February 2010 in two districts, Azadshahr and Ramian. Ten villages were selected by clustered random sampling across these two districts. The information on numbers and distributions of animal hosts was collected from local agriculture and horticulture office. The total number of livestock was 32413 including 14674 (45.2%) cows, 6529 (20%) sheep, 9349 (28.8%) goats and 30 (6%) camels. The desired sample size for the study was calculated using the 95% confidence interval and at 5% absolute precision ( $Z=2$ ,  $P=0.6$ ,  $d=0.044$ ). In total, 498 animals from 20 herds including 100 sheep, 143 goats and 225 cows, and also 30 camels from 5 herds of all sexes were selected randomly and examined individually for tick infestation. Ticks were collected at intervals of between 1 and 5 weeks within a season. Thirty minutes were spent for each flock to collect ticks. All inspections and tick collections were carried out between 08:00 a.m. and 11:00 a.m. In case of infestation, ticks were collected using forceps and then preserved in 70% ethyl alcohol. All collected specimens were preserved in tubes and relative information were recorded. Specimens were deposited at the Medical Entomology Laboratory of School of Public Health at Tehran University of Medical Sciences. The identification keys of Hoogstraal[12] and Walker *et al.*[13] were used to identify each tick to species.

Meteorological data from March 2009 through February 2010, including monthly minimum, mean and maximum temperatures, relative humidity, mean monthly rainfall and annual evaporation in the mountainous area of Golestan were obtained from Iran Meteorological Organization. The geographical points were recorded using a Garmin eTrex®H GPS. Other data including date, type of host, infested organ of hosts and number of ticks were recorded. Software ArcGIS, version 9.2 was used for mapping and the spatial analysis.

### 2.3. Statistical analysis

Linear regression was performed to analyze the relationship between presence of each tick species and environmental factors including mean monthly rainfall (mm), annual evaporation (mm), and mean annual temperature. A *Chi*-square test was used to compare the species distribution and seasonal data. For all comparisons,  $P<0.05$  was considered significant. The density of infestation was calculated as the total number of ticks observed divided by the number of infested host, and mean density of ticks per host was calculated as the total number of ticks observed divided by the total of host examined.

## 3. Results

### 3.1. Species diversity of ixodid ticks infesting cattle

A total of 255 ixodid ticks were found on 219 different hosts, including 44 sheep, 63 goats, 99 cows and 13 camels. Five

**Table 1**

Numbers and prevalence of ixodid ticks in ten different villages in mountainous area of Golestan province.

Villages	Coordination		Elevation (m)	<i>R. sanguineus</i>		<i>R. bursa</i>		<i>H. marginatum</i>		<i>H. anatolicum</i>		<i>H. asiaticum</i>		Total	
	N	E		No	%	No	%	No	%	No	%	No	%	No	%
Kashidar	36.59.13	55.33.16	1358	20	7.8	0	0.0	15	5.9	0	0.0	0	0.0	35	13.7
Vamenan	37.00.30	55.33.12	1380	18	7.1	2	0.8	0	0.0	0	0.0	0	0.0	20	7.8
Nodeh	37.04.27	55.15.42	209	14	5.5	7	2.7	5	2.0	0	0.0	0	0.0	26	10.2
Azadshahr	37.05.14	55.11.19	138	15	5.9	0	0.0	8	3.1	0	0.0	0	0.0	23	9.0
Nili	37.07.03	55.15.28	203	13	5.1	0	0.0	2	0.8	3	1.2	1	0.4	19	7.5
Nezamabad	37.08.10	55.09.59	89	12	4.7	0	0.0	6	2.4	10	3.9	4	1.6	32	12.5
Ghoorchay	37.09.48	55.03.57	47	17	6.7	0	0.0	15	5.9	2	0.8	5	2.0	39	15.3
Paghaleh	36.54.51	55.06.08	1155	30	11.8	0	0.0	0	0.0	0	0.0	0	0.0	30	11.8
Sheshab	36.55.53	55.07.32	487	20	7.8	0	0.0	0	0.0	0	0.0	0	0.0	20	7.8
Bagherabad	36.57.21	55.06.55	414	11	4.3	0	0.0	0	0.0	0	0.0	0	0.0	11	4.3
Total	–	–	–	170	66.7	9	3.5	51	20.0	15	5.9	10	3.9	255	100.0

N=North, E=East, No=number of collected ticks, %=percentage of ticks.

**Table 2**

Ixodid tick species infesting animal hosts in mountainous area of Golestan province, Iran, 2009–2010.

Host	Total surveyed host	Sex of infested host	Number of infested host	<i>R. sanguineus</i>		<i>R. bursa</i>		<i>H. marginatom</i>		<i>H. anatolicum</i>		<i>H. asiaticum</i>		Total	
				No	%	No	%	No	%	No	%	No	%	No	%
Sheep	100	Male	20	21	8.2	0	0.0	10	3.9	1	0.4	3	1.2	35	12.5
		Female	24	35	13.7	2	0.8	12	4.7	6	2.4	2	0.8	57	22.4
Goat	143	Male	21	29	11.4	3	1.2	5	2.0	0	0.0	2	0.8	39	15.3
		Female	42	56	22.0	4	1.6	14	5.5	0	0.0	1	0.4	75	29.4
Cow	225	Male	38	11	4.3	0	0.0	2	0.8	2	0.8	2	0.8	17	6.7
		Female	61	9	3.5	0	0.0	6	2.4	4	1.6	0	0.0	19	7.5
Camel	30	Male	4	4	1.6	0	0.0	1	0.4	0	0.0	0	0.0	5	2.0
		Female	9	5	2.0	0	0.0	1	0.4	2	0.8	0	0.0	8	3.1
Total	498		219	170	66.7	9	3.5	51	20.0	15	5.9	10	3.9	255	100.0

No= number of collected ticks on the hosts, %= percentage of ticks.

hard tick species from two genera were found to parasitize animal hosts in mountainous area of Golestan including *Rhipicephalus sanguineus* (*R. sanguineus*) (Latreille, 1806), *Rhipicephalus bursa* (*R. bursa*) (Canestrini and Fanzago, 1878), *Hyalomma marginatum* (*H. marginatum*) (Koch 1844), *Hyalomma anatolicum* (*H. anatolicum*) (Koch, 1844) and *Hyalomma asiaticum* (*H. asiaticum*) (Schulze and Schlottke, 1930) (Tables 1 and 2). *R. sanguineus* and *H. marginatum* were the most prevalent species in the study area.

### 3.2. Seasonal activity of ixodid tick infesting domestic ruminants

The seasonal activity of ixodid tick infesting domestic ruminants in the area was determined based on their relative abundance (species *v.s.* season). More than 35% and 46% of the total ticks were collected in spring and summer, while 13.3% and 5.2% were collected in autumn and winter of the study period respectively (Table 3). Tick infestation was at the highest rate in the summer, while their lowest infestation rate was in the winter. *R. sanguineus* as dominant species was active during the whole year, while *R. bursa* and *H. asiaticum* were collected only in spring and summer. *H. anatolicum* was not found in the winter during the study period (Table 3). Although the number of collected ticks and also infested animals varied in the

seasons, there was no statistically significance between the seasons and the presence of ticks ( $\chi^2=2.560$ ,  $df=6$ ,  $P=0.862$ ). However, the relative abundance of ticks were significant in two districts, Azadshahr and Ramian ( $\chi^2=31.622$ ,  $df=4$ ,  $P=0.001$ ).

**Table 3**

Seasonal activity of ixodid ticks species in the mountainous area of Golestan province, Iran, 2009–2010.

Season species	Spring		Summer		Fall		Winter		Total	
	No	%	No	%	No	%	No	%	No	%
<i>H. marginatum</i>	15	5.9	25	9.8	8	3.1	3	1.2	51	20.0
<i>H. anatolicum</i>	4	1.6	8	3.1	3	1.2	0	0.0	15	5.9
<i>H. asiaticum</i>	5	2.0	5	2.0	0	0.0	0	0.0	10	3.9
<i>R. sanguineus</i>	63	24.7	74	29.0	23	9.0	10	3.9	170	66.7
<i>R. bursa</i>	4	1.6	5	2.0	0	0.0	0	0.0	9	3.5
Total	91	35.7	117	45.9	34	13.3	13	5.1	225	100.0

No=number of collected ticks in the each season, %=percentage of ticks.

### 3.3. Host use and attachment sites

The mean densities of ticks per host were calculated as 2.02, 1.8, 0.36 and 1 on sheep, goats, cows and camels respectively. The densities of infestations were calculated as 0.9, 0.79, 0.16 and 0.43 for each mentioned host respectively. Based on the mentioned indicators sheep were the most infested animal host and cows were recorded

as the less infested host in this study. Sheep were infested with all five hard ticks while cows and goats were infested with four species and camels with only three species of ixodid ticks (Table 2). Hard ticks were infested in different parts of the host body including ear, mammary glands, under tail and the rest of body. The heaviest infestation was observed on ear 35.7%, followed by under tail 29.8%, mammary gland 21.6%, and body 12.9% (Table 4). The high infestation was recorded in *R. sanguineus* on ear of its hosts.

**Table 4**

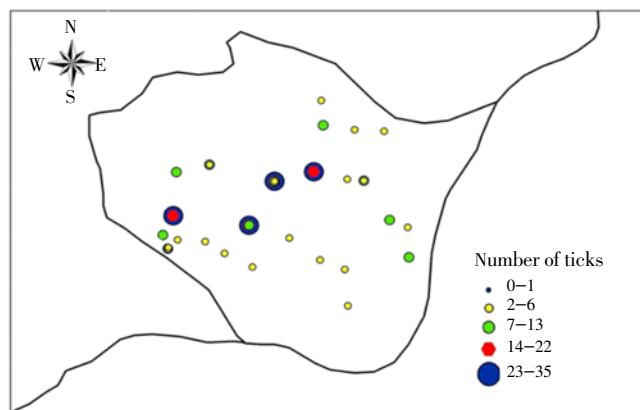
Body part infestation of Ixodid tick species on hosts in mountainous area of Golestan province, Iran, 2009–2010.

Species organ	<i>R. sanguineus</i>		<i>R. bursa</i>		<i>H. anatolicum</i>		<i>H. marginatum</i>		<i>H. asiaticum</i>		Total	
	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)
Ear	51	20.0	3	1.2	6	2.4	25	9.8	6	2.4	91	35.7
Mammary glands	40	15.7	1	0.4	2	0.8	10	3.9	2	0.8	55	21.6
Body	30	11.8	0	0.0	2	0.8	1	0.4	0	0.0	33	12.9
Under tail	49	19.2	5	2.0	5	2.0	15	5.9	2	0.8	76	29.8
Total	170	66.7	9	3.5	15	5.9	51	20.0	10	3.9	255	100.0

No=number of collected ticks on different parts of the host body, %=percentage of ticks.

### 3.4. Geographical distributions

The geographical distribution of ticks on their hosts is presented in Figure 2. *R. sanguineus* was the most prevalent species in all districts followed by *H. marginatum* as second dominant species. *R. bursa* had the lowest prevalence (3.5%) in this study (Tables 2 and 3).



**Figure 2.** Geographical distribution of ixodid ticks on domestic ruminants in mountainous area of Golestan province, 2009–2010.

The three species belong to genus *Hyalomma* had narrow distribution in the study area and were found only in three localities; while the two species belong to genus *Rhipicephalus* had wider distribution in the mountainous area. *R. sanguineus* was the most distributed ixodid tick across the area (Table 1). The lowest tick infestation was observed in Bagherabad with prevalence of 4.3% while, the highest was found in Ghoorchay with prevalence of 15.3%. Regression analysis (among monthly minimum, mean and maximum temperatures, relative humidity, mean monthly rainfall, annual evaporation and occurrence of each tick species) confirmed the absence of any relationship between

the tick species and the environmental factors. Since no statistically significant differences ( $P>0.05$ ) were observed between the tick species and the environmental factors in this area, the descriptive maps were produced to identify known distributions of ticks. The geographical distributions of tick species on hosts and distribution of *R. sanguineus*, as the most dominant species in the area are presented in Figures 2 and 3.



**Figure 3.** Geographical distribution of *R. sanguineus* in mountainous area of Golestan province, 2009–2010.

### 4. Discussion

A number of articles have been published on the ixodid ticks of Iran. Most of them focused on ticks of domestic and wild animals, their fauna and the pathogens they carry[3,14]. As ticks are very important vectors of human and animal diseases, they have been subject of many studies in Iran. To date, there is very limited information about geographical distribution of tick infestation in a certain geographical areas.

Results of the present study showed that five ixodid tick species from two genera infested domestic ruminants in mountainous area of Golestan province in 2009–2010. Previous studies have shown that these species were found in other parts of the country, some of them were found in the temperate and humid climate in neighboring provinces of Golestan, others in arid land of the country. Former studies, notably those by Nabian *et al.*[10] and Rahbari *et al.*[11], ascertained the occurrence of the genus *Haemaphysalis* in Golestan province, while it was not found in the study area. This could be due to the fact that the mountainous area has reasonably different climate compared to other parts of the province.

The brown dog tick, *R. sanguineus*, has widely distributed around the world and was found widely across the country[3]. It was the more frequent and abundant species with prevalence of 66.7% in this study, while Nabian and Rahbari found it with prevalence of 4.5% as second abundant species in Zagros mountainous area of Iran[14]. It was found with prevalence of 14.06% from Golestan province[10]. The result of this study is in agreement with



the results obtained from studies in South Khorasan, Ghaemshahr and Quazvin<sup>[9,15,16]</sup>. It was found that 50.5% of sheep were infested with *R. sanguineus* as a dominant tick in South Khorasan at the east of our study area<sup>[15]</sup>. Hosseini *et al.* in a similar study showed the dominance of this species (82.4%) in Ghaemshahr at the west of our study area<sup>[16]</sup>. *R. sanguineus* was indicated as the most abundant ixodid species (46.92%) on sheep in Quazvin province<sup>[9]</sup>. Although, it is known as the common tick parasite of dog, it can also feed on other animals and man<sup>[17,18]</sup>. Comparison of host preference of *R. sanguineus* revealed that 7.8%, 22%, 33.3% and 3.5% were parasite of cows, sheep, goats and camels respectively. Sheep were the most preferred host of this species throughout the study area. *R. sanguineus* was dominant species from aspects of the abundance, dispersal and infestation of domestic ruminant across the study area. This can be attributed to the fact that the species has wide distribution and can tolerate adverse climatic and environmental conditions.

Although *R. bursa* distributed over the country, it was the infrequent species with prevalence of 3.5% in the mountainous area of Golestan, which is in agreement with the result of a survey in Ghaemshahr<sup>[16]</sup>. In contrast, Nabian and Rahbari found this species with prevalence of 21.9% as the most abundant species in Zagrous mountainous area of the country<sup>[14]</sup>, while it was the second abundant species in Mazandaran province<sup>[8]</sup>. In another study, *R. bursa* was found with prevalence of 9.37% from Golestan province<sup>[10]</sup>. Shemshad *et al.* found *R. bursa* with prevalence of 2.2% among six species in Quazvin province<sup>[9]</sup>. Although *R. bursa* was recorded as the infrequent species in our study area, it was the only species which was found in Bagherabad with elevation of more than 3000 m above the sea level.

*H. marginatum* was the second abundant species (19.9%), while it was recorded as dominant species in Golestan, Abdanan and Zagrous mountainous area with prevalence of 54.68%, 44.6% and 13% respectively<sup>[14,19]</sup>. This species was recorded as the most dominant species in Zagrous mountainous area; it was also found in Mazandaran and Yazd provinces with more or less the same prevalences (13.2% and 12.5% respectively)<sup>[8,20]</sup>.

*H. anatolicum* and *H. asiaticum* were found with prevalence of 5.9% and 3.9% respectively, which are in agreement with the results obtained from studies in Mazandaran and Yazd provinces<sup>[8,20]</sup>. *H. anatolicum* was the infrequent species with prevalence of 0.4% among six ixodid species in Quazvin province<sup>[9]</sup>, while it was the second abundant tick infesting sheep (48.5%) in south Khorasan on the east of Golestan province<sup>[9]</sup>.

This study showed that all animal hosts were infested to hard ticks. The overall mean density of ticks per host and the overall density of infestation were 1.16 and 0.51 respectively. Based on the calculated average number of ticks per each host and also density of infestation, sheep were the most infested animal host and cows were recorded as the lowest infested host in this study. Infestation rate in female sheep (2.38) were 7.6 times more than female cows (0.31). Although there was a seasonal variation in the tick infestation, there was no significance between the seasons and the presence of ticks ( $P < 0.05$ ). However, some species including *R. sanguineus* and *H. marginatum* did occur throughout the year, but others were found in certain seasons. The prevalence of ticks in spring and summer were at the highest rate (81.6%) while it was in the lowest

rate in the autumn and winter (18.4%). The result is slightly different from the result obtained from a study form Ilam in the western part of Iran. Nasiri *et al.* indicated that the prevalence of ticks on animal hosts was at the highest rate in spring and autumn, while in the summer and winter were at the lowest rate<sup>[19]</sup>. It seems that this is because of different geographical, metrological, sampling method and the diversity of ixodid ticks in Ilam and mountainous area of Golestan. The moderate climate, ever green plant or year-long vegetation and abundant livestock hosts in the area provide suitable habitats for these ticks.

Geographical information system has been used with analytical and descriptive approach to determine tick distributions and also for modeling their occurrence in space and time. Such information including predictive maps are very useful tools for health authorities in order to monitor and control tick and tick-borne diseases. Although, in this study there were no significant differences between tick species and the environmental factors, but the descriptive maps provided. The reason could be due to the movement of flocks from mountainous area to lowlands in different seasons by rural people and shepherds. As three different zones are recognized in Golestan province, therefore we suggest conducting a similar research in lowlands and coastal areas of the province. However, the result of this study is considered as the essential starting points to generate distribution maps for ticks and their host in the mountainous area of Golestan.

*H. anatolicum* and *R. sanguineus* have been incriminated as vector of Crimean–Congo hemorrhagic fever in Hamadan, Iran<sup>[21]</sup>. Recently, Salimabadi *et al.* have shown five infected tick species from *Hyalomma* genus to Crimean–Congo hemorrhagic fever virus<sup>[20]</sup>. *H. marginatum*, *H. anatolicum* and *H. asiaticum* were among the infected ticks in Yazd, in the central plateau of the country. These documents show the important role of hard tick in human health. It is important to understand the geological distribution of medically and veterinary important ticks in order to apply effective tick control program and prevention of the disease in the region. The distribution and abundance of hard ticks as vector of man and animal diseases are very important issue from epidemiological point of view. Such research provides necessary information for human and animal health service managers to have a better understanding of prevention and control of vector borne diseases especially during the outbreaks.

### Conflict of interest statement

We declare that we have no conflict of interest.

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## Comments

### Background

Ticks are ectoparasitic arthropods that may cause severe reductions on health status and productions of livestock. In addition, ticks are the vectors of important viral, bacterial and parasitic pathogens that are the causative agent of important human or veterinary diseases.

### Research frontiers

The present research work aimed to evaluate prevalence and geographical distribution of ticks on livestock (sheep, goats, cows and camels) in the mountainous areas of Golestan province, Iran and to identify isolated ticks at the species level.

### Related reports

All tick species isolated in the present study were already reported in Iran and in other areas of Golestan. Furthermore, diseases and economic losses or vector-borne diseases directly caused or transmitted by ticks isolated in this study were already reported in Iran.

### Innovations and breakthroughs

In the present study, authors evaluated prevalence and species distribution of hard ticks in the highland regions of Golestan, Iran, an area never considered before. Mapping and intensity of ixodid ticks infestation on domestic ruminants and seasonal activity of isolated tick species were also evaluated.

### Applications

Previous data on species, prevalence and distribution of ticks in the considered area are lacking. In addition, results on the identification, prevalence, distribution, intensity, and seasonal activity of tick species isolated from domestic ruminants obtained in the present study could be extremely useful for the control of ticks and tick-borne diseases in the considered area.

### Peer review

This is an interesting and good research work in which authors evaluated prevalence, distribution, intensity and seasonal activity of ticks isolated from domestic ruminants in the highland regions of Golestan, Iran. Collected ticks were identified at the species level, counted and geo-referenced. In addition, isolated ticks were related to animal species, season and environmental data recorded during the study period. The results seem really interesting and useful for the control of ticks and tick-borne diseases in the considered area.

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