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A survey on species diversity and distribution of hard ticks (*Acari: Ixodoidea*) in cattle and buffaloes, Miandoab, Iran

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ABSTRACT

The family of *Ixodidae* is one of the greatest tick families of domestic animals that includes 13 genera and 650 species in five subfamilies. The goal of this research is the assessment of the cattle and domestic buffalos' contaminations development to differentiate the tick family from the quantitative aspect and also the determination of the found tick species diversity in Miandoab. In this study, conducted from the early spring 2016 to the late winter in 2017, 406 ticks were collected and identified from the total cattle and buffaloes studied; 187 ticks were collected and identified. From all cattle studied, *Hyalomma anatolicum anatolicum*, with 179 numbers (44.09%), was identified as the most infesting agent, and *Boophilus annulatus*, with three numbers (0.74%), was identified as the least infecting agent. From the total buffaloes under studied, *Hyalomma anatolicum anatolicum*, with 101 numbers (54.01%), was identified as the highest cause of infection, and *Rhipicephalus sanguineus*, with 21 numbers, (11.23%) was identified as the least infecting agent (P<0.05). In the current study, in terms of seasonal prevalence of hard tick infection, the highest level of infection was observed in spring and the lowest levels of infection were observed in winter (P<0.05). Maximum rates of hard ticks were observed in the groin and minimums were observed on the surface of the testicles (P<0.05).

بررسی تنوع گونه و توزیع کنه های سخت (*آکاری: ایکسودیده*) گاو و گاومیش های شهرستان میاندوآب، ایران

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چکیده

خانواده *ایکسودیده* یکی از بزرگ‌ترین خانواده‌های کنه‌های حیوانات اهلی است که شامل ۱۳ جنس و ۶۵۰ گونه در پنج زیر خانواده است. هدف از این تحقیق، بررسی شیوع آلودگی گاو و گاومیش های شهرستان میاندوآب به گونه‌های مختلف کنه‌های سخت از نظر کمیت و همچنین شناسایی تنوع گونه‌های کنه‌ها می‌باشد. در این مطالعه که از ابتدای بهار ۱۳۹۵ تا پایان زمستان سال ۱۳۹۶ انجام شد، ۴۰۶ کنه از کل گاوها و ۱۸۷کنه از کل گاومیش های مورد مطالعه، جمع آوری و شناسایی شدند. از مجموع گاوهای مورد مطالعه، *هیالوما آناتولیکم آناتولیکم* با ۱۷۹ عدد (۴۴/۰۹٪) به عنوان بیشترین عامل آلوده کننده و *بوآفیلوس آنولاتوس* با سه عدد (۰/۷۴٪) به‌عنوان کم‌ترین عامل آلوده‌کننده شناخته شدند. از مجموع گاومیش های مورد مطالعه، *هیالوما آناتولیکم آناتولیکم* با ۱۰۱ عدد (۵۴/۰۱٪) به عنوان بالاترین عامل آلوده کننده و *رپی سفالوس سانگوینوس* با ۲۱ عدد (۱۱/۲۳٪) به عنوان کمترین عامل آلوده کننده شناخته شدند (P<۰/۰۵). در مطالعه حاضر، از نظر شیوع فصلی آلودگی کنه سخت، بیشترین میزان آلودگی در فصل بهار و کمترین میزان آلودگی در فصل زمستان مشاهده شد (P<۰/۰۵). بیشترین میزان کنه های سخت در کشاله ران و کمترین آن در سطح بیضه مشاهده شد (P<۰/۰۵).

واژه های کلیدی: کنه سخت، *ایکسودیده*، گاو، گاومیش، میاندوآب

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INTRODUCTION

Ticks are hematophagous ectoparasites that are known as pests and vectors of a wide range of diseases of humans, livestock, pets, and wild animals. It has been estimated that about 80% of the world buffaloes' population is infested with ticks [1]. Ticks have a variety of direct and indirect effects on their hosts. Tick infestations can cause considerable irritation in animals and can lead to severe disorders, such as blood loss, general stress, damage to the skin, tick paralysis, and tick toxicosis [2,3]. In the Middle East, taxonomically accurate information on tick species is limited, and the tick-borne diseases of this region remain poorly characterized. The relationship between ticks and tick-borne pathogens in the region is largely unknown, even though the presence of these pathogens has been recognized for many years and the number of new pathogens discovered in ticks has increased markedly [4-6]. Advances have recently been made in Middle East infectious disease research, but vector-borne diseases are still misdiagnosed and underestimated because of inadequate clinical training and limited surveillance and laboratory capacity. Tick species that occur in Iran have been discussed by Toumanoff in 1944 [7], Hoogstraal et al. and Wilson between the 1960s and 1980s [8-13], Petney and Keirans in the mid-1990s [14-17], Robbins et al. in 1996 [18], and Kernif et al. in 2012 [4] the most recent work.

Cattle and Buffaloes are reared in Miandoab by releasing them into the forest and by periodically moving them close to or in the pen. Such practices may facilitate interactions between ticks and their hosts, including the exchange of ticks between wild and domestic animals. They may also lead to dispersal of the tick population, thereby potentially increasing the risk of tick-borne disease transmission. The goal of this research is the assessment of cattle and buffalo contamination development to differentiate tick families from the quantitative aspects and also determination of the found tick species diversity in Miandoab.

MATERIALS AND METHODS

Study area

The present study was performed in 48 different rural areas of Miandoab (West Azerbaijan province, northwest, Iran, 37°23′21″N 46°14′15″E). During a year, 12 sampling periods (one period every month) were referenced to 48 designated geographical areas, so that at each stage 4 regions (one of each west, east, north and south areas) were examined.

Sample size

In this study, a total number of 480 cattle and 240 buffaloes of different age groups (less than 1 year, 1–2 years old, 2–3 years old, and over 3 years old) were selected by stratified random sampling over the course of 1 year (Oct 2015 to Sep 2016). At each stage, 8 samples were randomly selected for each of the four study areas. The examined buffaloes and cattle were raised under traditional husbandry practices (grazing on pastures during the day) without regular acaricide treatment. A total of 187 ticks were collected from Buffaloes and 406 ticks were collected from cattle. Data for all specimens, including date, sex, age, and the number of ticks, were recorded. All of the methods used in this study were confirmed by the Ethics Committee of Islamic Azad University of Urmia, respecting currently accepted animal welfare rules in accordance with the ethical standards of the responsible committee on human

experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008.

Parasitological procedures

At first, the animal was fastened before any inspections, then different parts of the body were examined. Due to the location of most ticks in the animal's low-lying areas, the perineal region, groin, axillary region, udder, genital organs and buffalo neck were inspected. Ticks were removed from the host with rubbing alcohol pads surrounding the skin and blunt pointed forceps, avoiding damage to the mouthparts of the ticks and the skin of the host [20]. The collected specimens were transferred into preserving tubes containing 70% ethanol (Merck, Darmstadt, Germany) and transferred to the Parasitology Research Laboratory of Islamic Azad University, Urmia, Iran. Following examinations under a stereomicroscope, ticks were identified by morphological characteristics using the key identification guide [21]. In some samples, 5% KOH solution was used to remove sediment and clarification.

Statistical analysis

For data analysis, descriptive statistics for qualitative data with 95% confidence intervals (95% CI) were used and logistic regression was used to determine the effect of mentioned risk indicators (sex, different parts of the body and season) on the prevalence of infection. Data were analyzed using Stata, version 11.2 (College Station, TX, USA).

RESULTS

According to the results, 98 of the 480 cattle (20.42 %) including 35 (35.71%) male and 63 (64.29%) female, which is a total of 406 ticks from infected cattle were identified. 36 of the 240 buffaloes (15.00 %) including 11 (30.55%) males and 25 (69.44%) females were infected and a total of 187 ticks from infected buffaloes were identified. Infesting ticks detected in cattle included *Hyalomma anatolicum anatolicum, Hyalomma anatolicum excavatum, Hyalomma detritum, Rhipicephalus bursa, Rhipicephalus sanguineus, Dermacentor marginatus,* and *Boophilus annulatus.*

Among these, *Hyalomma anatolicum anatolicum* (44.09%) was identified as the most infecting agent, and *Rhipicephalus bursa* (17.24%), *Hyalomma anatolicum excavatum* (13.30%), rhipicephalus sanguineus (12.07%), *Hyalomma detritum* (8.13%), *Dermacentor marginatus* (4.43%) and *Boophilus annulatus* (0.74%) had the next rates, respectively. The frequency of different species of hard ticks in cattle is shown in Table 1 and Figure 1.

The diversity of infesting ticks identified in buffaloes included *Hyalomma anatolicum anatolicum, Hyalomma anatolicum excavatum, Rhipicephalus bursa,* and *Rhipicephalus sanguineus*. Among them, *Hyalomma anatolicum anatolicum* (54.01%) was identified as the most infecting agent, and *Rhipicephalus bursa* (21.93), *Hyalomma anatolicum excavatum* (12.83%) and *Rhipicephalus sanguineus* (11.23%) had the next rates, respectively. The frequency of different species of hard ticks in buffaloes is shown in Table 2 and Figure 2.

Table 1. The prevalence of tick species in examined cattle in Miandoab, Iran

Hard Tick Species	Frequency of Infected Cattle						Frequency of Hard Tick							
	Male		Female		Total		M. Mature		F. Mature		Nymph&Larvae		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
H.anatolicum anatolicum	14	40.00	25	39.68	39	39.79	82	49.69	86	38.57	11	61.11	179	44.09
H.anatolicum excavatum	4	11.43	7	11.11	11	11.22	17	10.30	33	14.80	4	22.22	54	13.30
H. deteritum	1	2.86	7	11.11	8	8.16	4	2.42	29	13.00	-	0.00	33	8.13
R. bursa	11	31.42	13	20.63	24	24.48	35	21.21	32	14.35	3	16.67	70	17.24
R. sanguineus	4	11.43	7	11.11	11	11.22	23	13.94	26	11.66	-	0.00	49	12.07
D. marginatus	1	2.86	3	4.76	4	4.09	4	2.42	14	6.28	-	0.00	18	4.43
B. annulatus	-	0.00	1	1.59	1	1.02	-	0.00	3	1.35	-	0.00	3	0.74
Total	35	35.71	63	64.29	98	100	165	40.64	223	54.93	18	4.43	406	100

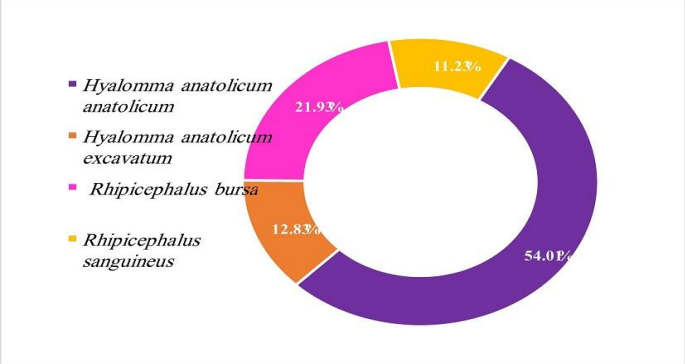

Figure1. Relative frequency of identified ticks in cattle in Miandoab, Iran

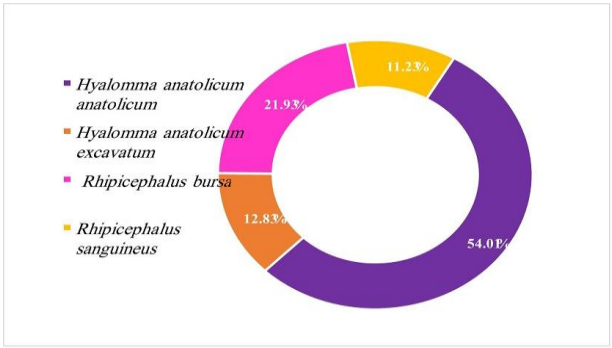

Figure 2. Relative frequency of identified ticks in buffaloes in Miandoab, Iran

Table 2. The prevalence of tick species in examined buffaloes in Miandoab, Iran

Hard Tick Species	Frequency of Infected Buffaloes						Frequency of Hard Tick							
	Male		Female		Total		M. Mature		F. Mature		Nymph&Larvae		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
H.anatolicum anatolicum	5	45.45	10	40.00	15	41.66	43	57.33	55	50.93	3	75.00	101	54.01
H.anatolicum excavatum	2	18.18	5	20.00	7	19.44	9	12.00	15	13.89	-	0.00	24	12.83
R. bursa	3	27.27	6	24.00	9	25.00	18	24.00	22	20.37	1	25.00	41	21.93
R. sanguineus	1	9.10	4	16.00	5	13.89	5	6.67	16	14.81	-	0.00	21	11.23
Total	11	30.56	25	69.44	36	100	75	40.11	108	57.75	4	2.14	187	100

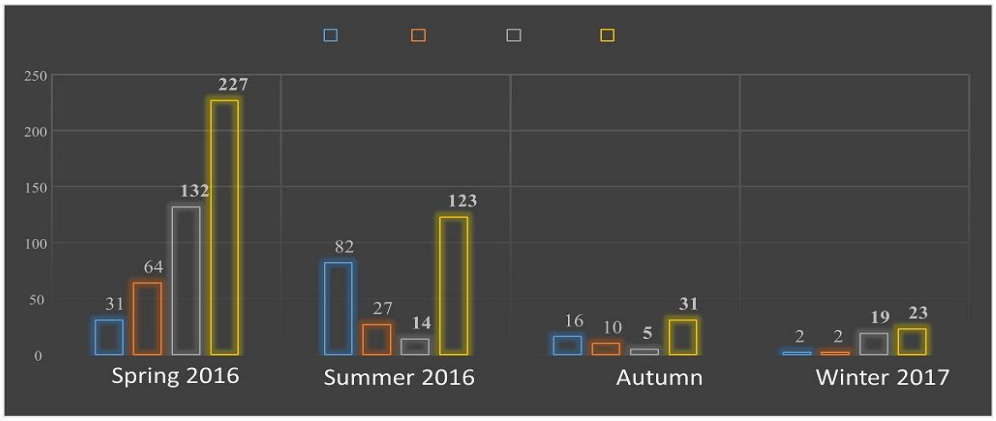

Figure 3. Total count of hard ticks infesting cattle in different months of the year in Miandoab, Iran

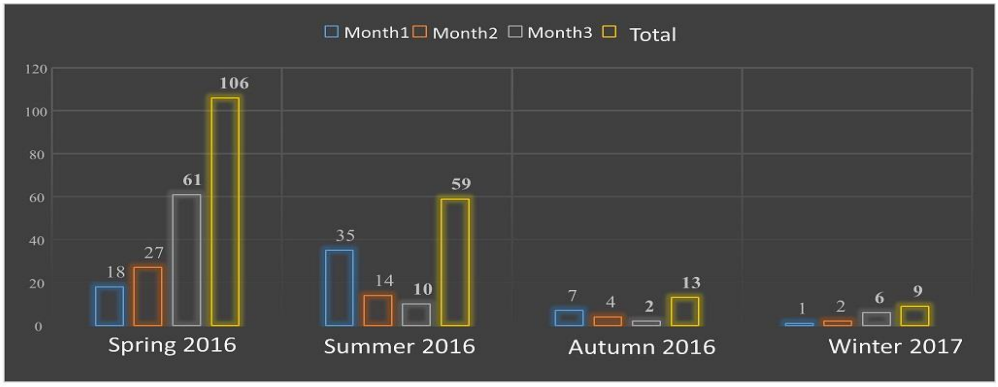

Figure 4. Total count of hard ticks infecting buffaloes in different months of the year in Miandoab, Iran

Table 3. Total count and relative frequency of identified hard ticks in cattle in different parts of the body of cattle in Miandoab, Iran

	Different Parts of the Body									
Hard Tick Genus	Frequency		Stratum		Udder		Perineum		Groin	
	No.	%	No.	%	No.	%	No.	%	No.	%
Hyalomma.spp	266	65.52	19	7.14	39	14.67	97	36.47	111	41.73
Rhipicephalus.spp	137	33.74	7	5.11	21	15.32	54	39.42	55	40.14
Boophilus.spp	3	0.74	0	0.00	0	0.00	3	100.00	0	0.00
Total	406	100.00	26	6.40	60	14.78	154	37.93	166	40.89

Table 4. Total count and relative frequency of identified hard ticks in buffaloes in different parts of the body of buffaloes in Miandoab, Iran

	Different Parts of the Body									
Hard Tick genus	Frequency		Stratum		Udder		Perineum		Groin	
	No.	%	No.	%	No.	%	No.	%	No.	%
Hyalomma.spp	125	65.52	6	4.80	12	9.60	39	31.20	68	54.40
Rhipicephalus.spp	62	33.74	3	4.84	7	11.29	19	30.64	33	53.52
Total	187	100.00	9	4.81	19	10.16	58	31.02	101	54.01

In current survey, from seasonally contamination prevalence aspect in cattle, in spring 227 (55.91%) ticks, in summer 123 (30.30%) ticks and in autumn and winter 31 (7.64%) and 23 (5.66%) ticks respectively, were found on cattle and the maximum contamination of cattle in spring and the minimum in winter were observed (Figure 3).

Among different parts of the body surface of cattle, groin with the average of 40.89% had the highest diversity and average count with different types of ticks and the stratum with the average of 6.40% had the lowest diversity and average count of ticks (Table 3).

In this survey, from seasonally contamination prevalence aspect in buffaloes, in spring 106 (56.68%) ticks, in summer 59 (31.55%) ticks and in autumn and winter 13 (6.95%) and 9 (4.81%) ticks respectively, were found in buffaloes and maximum contamination of buffaloes in spring and minimum in winter were observed (Figure 4).

From total under-studied buffaloes, groin with 54.01% average like cattle had the highest variety and average count of ticks and the stratum with 4.81% average like cattle studied had the least variety and frequency of ticks (Table 4).

DISCUSSION

Miandoab is one of the most populated areas in livestock farming in terms of cattle and buffalo populations. Ticks are one of the most important pests in the livestock industry and have the ability to transmit various diseases. Of the various diseases transmitted by ticks, some such as theileriosis and babesiosis are widespread in the region. Therefore, a study on the diversity of species of ticks of cattle and buffaloes in Miandoab was done to obtain more epidemiological dimensions of diseases caused by ticks in cattle and buffaloes of this region. Also, considering that a lot of research is being done on making anti-tick vaccines, it is important to identify the tick species of each region [9]. According to the results obtained in this study, the species diversity of the found ticks in cattle were *Hyalomma anatolicum anatolicum* (44.09%) and *Hyalomma anatolicum excavatum* (13.30%), *Hyalomma deteritum* (8.13%), *Rhipicephalus bursa* (17.24%), *Rhipicephalus sanguineus* (12.07%), *Boophilus annulatus* (0.74%) and *Dermacentor marginatus* (4.43%). Among the total studied buffaloes, the species diversity of the found ticks was *Hyalomma anatolicum anatolicum* (54.01%), *Hyaloma anatolicum excavatum* (12.83%), *Rhipicephalus bursa* (21.93 %), *Rhipicephalus sanguineus* (11.23%). The results of this study indicate the absolute dominance of the genus *Hyalomma* and especially the species of *Hyalomma anatolicum anatolicum*. According to the results of a study conducted by Mazlum et al., in 1971, *Hyalomma anatolicum anatolicum*, *Rhipicephalus bursa*, and *Rhipicephalus sanguineus* were mentioned as the most common ticks in Iran, which are widely spread in different parts of the country [7].

In another study on the species diversity of ticks in Tabriz and its suburbs, conducted by Hashemzadeh in 2006, *Hyalomma anatolicum* tick had the highest infection, followed by *Hyalomma anatolicum*, ticks of the genus *Rhipicephalus* (*Rhipicephalus sanguineus* and *Rhipicephalus bursa*), They had more contaminations [5]. The results of this study in Miandoab introduced *Hyalomma anatolicom* as the most common tick infecting cattle and buffaloes, which is consistent with the results of Nematollahi and Asadi who introduced the genus *Hyalomma* as the most common tick in Marand and suburbs [2]. In the study of species diversity of ticks in Garmsar, which was done by Bahadori et al., in 2002, *Rhipicephalus* ticks are the most infected (71.93%) in Garmsar [14]. In another study by Bahrami et al. in West Azerbaijan, the highest percentage of infection with hard ticks was related to *Rhipicephalus* (42%) and the lowest was related to *Haemaphysalis* (3%) [3]. *Rhipicephalus bursa* tick and *Rhipicephalus sanguineus* are also very important ticks in Iran and play a role in transmitting important pathogens to livestock in this regard, such as *Babesia Canis*, *Babesia Aqui*, *Babesia Caballi*, *Theleria Ovis*, *Anapalsma marginale* and *Rickettsia* [12, 13]. The survey showed that *Rhipicephalus sanguineus*, like *Rhipicephalus bursa* tick, has spread widely in Miandoab city and suburbs, and its prevalence has been reported in Khorasan Province in Razmi et al. studies. Therefore, this finding of Razmi et al. could indicate the greater prevalence of *Rhipicephalus sanguineus* in the country, contrary to the oppressed belief, because he mentioned the spread of *Rhipicephalus sanguineus* tick mainly was limited to the southern and southeastern regions of Iran [1]. In the study based on seasonal prevalence in cattle, out of the 406 hard ticks identified in this study, which infected 98 cattle, the highest level of infection was recorded in spring

(55.91%) and the lowest level of infection was recorded in winter (5.66%). Of the total studied buffaloes, out of the 187 hard ticks identified in this study, which infected 36 buffaloes, the highest level of infection was recorded in spring (56.68%) and the lowest level of infection was recorded in winter (4.81%). The results show that the head, ears, groin, axilla, and perineum are more exposed to infecting ticks than other anatomical parts of the body [4].

CONCLUSION

In this study, the distribution of hard tick was in the different parts of the body of cattle and buffaloes, and according to the results, ticks related to the genus *Hyalomma* and *Rhipicephalus* were found in all four parts of the body of the cattle and buffaloes. Among the four surfaces of the body, the groin with infesting rate of 40.89% in cattle and the average of 54.01% in buffaloes, had the highest diversity and infection with different types of ticks. The stratum in cattle with the average of 6.40% and in buffaloes with the average of 4.81%, had the lowest diversity and infection with different types of ticks.

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ETHICS

Approved.

CONFLICT OF INTEREST

There is no conflict of interest between the authors.

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