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Original article

IXODES TICKS AND CASES OF BRUCELLOSIS IN TAVUSH PROVINCE OF ARMENIA: SUSTAINABLE AGRICULTURE

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Abstract

Background. Ticks are known to cause tick-borne zoonotic diseases (meta-zoonosis). On the other hand, several factors, such as animal gender and animal replacement, pet dogs/their owner associations as well as soil composition, have been reported to be potential risk factors for brucellosis at the animal level. Currently, scientific data is also being accumulated that proves the association between *Ixodes* ticks and brucellosis cases.

Purpose. The current study in the range of "The role of *Ixodes* ticks in the transmission of brucellosis" aims to study the prevalence of *Ixodes* ticks in Tavush Province of Armenia in association with the loci of outbreaks of brucellosis in Tavush.

Materials and methods. Fieldwork was conducted from January to December 2023 in the Berd Region of the Tavush Distinct. Ticks were gathered/quantified/prepared for analysis according to standard methodological guidelines. Flagging was conducted at intervals of 50–60 steps, yielding approximately 30 samples per geographic zone.

For the database creation on animal brucellosis cases during 1950-2020, archival data from relevant organizations and internet sources were studied.

Results. Through a comprehensive study, the occurrence of six tick species in the Berd region with diverse ecosystems, including plains, forests, subalpine, and alpine zones – *Rhipicephalus annulatus, Rhipicephalus bursa, Ixodes ricinus,*

Dermacentor marginatus, Hyalomma marginatum and *Hyalomma scupense* – was documented.

Conclusion. The results of the present studies do not exclude the role of *Ixodes* ticks in the outbreaks of brucellosis cases in the region. The results are important for the development of strategies for zoonosis control as well as for the determination of pastures for livestock.

Keywords: livestock; geographical zones; Tavush province; Ixodes; brucellosis

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Научная статья

ИКСОДОВЫЕ КЛЕЩИ И СЛУЧАИ БРУЦЕЛЛЕЗА В ТАВУШСКОЙ ОБЛАСТИ АРМЕНИИ: УСТОЙЧИВОЕ СЕЛЬСКОЕ ХОЗЯЙСТВО

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Аннотация

Обоснование. Известно, что клещи вызывают клещевые заболевания животных (метазоонозы). С другой стороны, возможными факторами риска распространения бруцеллеза на уровне животных являются пол животных и перемещения животных, взаимоотношения собака-хозяин, а также состав почвы. В настоящее время также имеются научные данные, позволяющие предположить связь между иксодовыми клещами и вспышками бруцеллеза.

Цель. Целью исследования было изучение распространенности иксодовых клещей в очагах вспышек бруцеллеза в Тавушской области Армении.

Материалы и методы. Полевые работы проводились в январе-декабре 2023 года в Тавушской области в районе Берд. Клещей собирали/определяли количественно и анализировали в соответствии со стандартными методическими указаниями. Отметки ставились с интервалом 50–60 шагов, для каждой географической зоны примерно 30 образцов.

Для создания базы данных по бруцеллезу животных с 1950 по 2020 год были изучены архивные данные профильных организаций и интернет-источников.

Результаты. В результате комплексного изучения равнинной, лесной, субальпийской и альпийской зон района Берд обнаружено шесть видов клещей: *Rhipicephalus annulatus, Rhipicephalus bursa, Ixodes ricinus, Dermacentor marginatus, Hyalomma marginatum и Hyalomma scupense.*

Заключение. Результаты проведенных исследований не исключают роли иксодовых клещей в вспышках бруцеллеза в регионе. Результаты важны для разработки стратегии борьбы с зоонозами, а также для определения пастбищ для скота.

Ключевые слова: животноводство; географические зоны; Тавушская область; иксодовые клещи; бруцеллез

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Introduction

Ticks and tick-borne diseases represent a substantial threat to both human and animal health, inflicting significant economic losses on the agricultural sector annually, partly due to the absence of preventative measures [33]. *Ixodes* ticks are temporary external parasites on vertebrates, predominantly mammals, and to a lesser degree, birds and reptiles [36]. The severity of tick infestations in animals can vary widely, with some animals hosting anywhere from a few to several thousand ticks. *Ixodes* ticks are also vectors for the pathogens of piroplasmid diseases, capable of transmitting these pathogens across all tick life stages. Both nymphal and adult stages of ticks can transmit pathogens through their eggs, via transovarial and transtadial pathways [4].

Ticks can transmit viruses transgenerationally, meaning they can carry pathogens throughout their entire lifespan [17]. *Rhipicephalus bursa* (*R. bursa*), a common tick parasite of ungulates, is prevalent across various geographical zones, particularly in warm, temperate subtropical regions. From an epidemiological perspective, it is considered to have a “mild” impact on the transmission of bacterial diseases [35]. *Hyalomma marginatum* (*H. marginatum*) may act as a vector for certain tick-borne diseases affecting humans and animals [27]. *Rhipicephalus annulatus* (*R. annulatus*) primarily targets cattle, though horses and goats can also be susceptible to infestation by this tick species [21].

Hyalomma scupense (*H. scupense)*, found in parts of North Africa, Asia, and Southern Europe, may serve as a natural vector for tropical bovine theileriosis [30]. The ticks *Ixodes ricinus* (*I. ricinus*) and *Dermacentor reticulatus* (*D. reticulatus*) play significant roles as carriers and vectors of various pathogens.

The quantitative distribution of ticks is influenced by environmental conditions and is crucial for understanding the dynamics of tick populations. The density of ticks in a given area can vary depending on the quality of the habitat and the availability of blood meals from hosts. Ticks exhibit considerable resilience to adverse environmental conditions [26].

Scientific data is also accumulating that proves a possible link between spreading ticks and other zoonotic diseases, such as brucellosis [34; 38]. From a biosecurity point of view, it requires not only a separate characterization of the distribution of ticks in ecosystems but also a discussion of that distribution depending on the foci of zoonotic diseases different from tick-borne zoonotic diseases.

The Berd Region in Tavush Province, located in the northeastern part of the Republic of Armenia, features region vertical geographical zones, including plains, forests, subalpine, and alpine zones. This region is noted for its rich and unique biodiversity and forested landscapes. The highest point in the region is Miapor, reaching an elevation of 2993 meters, while the lowest point is the Varagajur River valley at 530 meters. The climate varies from mild subtropical dry conditions in the river valleys and lowlands to cooler temperatures and increased precipitation with elevation. This climatic diversity, coupled with rich forests and vegetation, provides favorable conditions for the proliferation and development of a wide range of fauna, particularly pasture ticks [14].

Current investigations in the range of "The role of *Ixodes* ticks in the transmission of brucellosis" aim to study the prevalence of *Ixodes* ticks in Tavush distinct of Armenia in association with the loci of brucellosis outbreaks in Tavush.

Purpose. The current study in the range of "The role of *Ixodes* ticks in the transmission of brucellosis" aims to study the prevalence of *Ixodes* ticks in Tavush.

Materials and methods

This research received approval from the Ethics Committee of the Armenian National Agrarian University on December 17, 2023.

Fieldwork was conducted from January to December 2023 in the Berd Region of the Tavush distinct. Ticks were gathered, quantified, and prepared for

analysis following established methodological guidelines using a 60 x 100 cm flag of thin cotton fabric [28]. Flagging was conducted at intervals of 50–60 steps, yielding approximately 30 samples per geographic zone. Adult tick species were identified using determinants by Pomarantsev, Serdyukova, and Filippova [16; 39], with observations made through a magnifying glass and an MBS-1 microscope. Statistical analyses were executed using the BioStat-2009 software.

For the database creation on animal brucellosis during 1950-2020, the following sources were used: National Archives of Armenia, Archive of the RA Ministry of Health, Archive of the RA Ministry of Agriculture, Sanitary and Epidemiological Station of the Armenian SR, Republican Anti-Plague Station, Yerevan Institute of Epidemiology and Hygiene Research Institute of Epidemiology, Microbiology, Parasitology, and internet resources.

Results

The quantitative distribution of pasture ticks across different geographic zones within the Berd region

Table 1 outlines the quantitative distribution of pasture ticks across different geographic zones within the Berd region for each season. Findings indicate a decline in tick populations with increasing altitude. Notably, no pasture ticks were identified across any of the four zones during January and February. The distribution of ticks was as follows: 52.1 % in the plain zone, 37.5 % in the forest zone, 8.4% in the subalpine zone, and a mere 2.0 % in the alpine zone. Furthermore, no ticks were found in the alpine and sub-alpine zones during March, April, and December, nor in the alpine zone in May and November, likely due to the low temperatures unfavorable for tick development.

The monthly average tick counts from the environment were: March - 41.75, April - 59.5, May - 146, June - 105.5, July - 105.5, August - 134.75, September - 142.25, October - 64.25, November - 31.75, and December - 10.5 (Table 1). These data align with prior research findings [3]. Among the species researched – *R. annulatus, I. ricinus, R. bursa, D. marginatus, H. scupense*, and *H. marginatum* – the *R. annulatus* species was the most prevalent, and *I. ricinus* the least common (accordingly 84.5 ± 13.69 and 161.2 ± 15.2 vs. 4.1 ± 0.62 and 2.2 ± 0.17; $P_1 < 0.05$). *D. marginatus* ticks predominantly inhabited subalpine and alpine zones, constituting 40.0 % and 59.7 % of the tick population in those areas, respectively. *R. annulatus* and *R. bursa* species were absent in the alpine zone, as were *H. scupense* ticks in the subalpine zone.

Table 1.

Quantitative prevalence of pasture ticks in geographical zones of the Berd Region, Tavush Province, (number, %*)

Observation time months, 2023	SUM	Plain Zone	Forest Zone	Subalpine zone	Alpine zone
January	-	-	-	-	-
February	-	-	-	-	-
March	167	116 (69.5)	51 (30.9)	-	-
April	238	169 (71.0)	69 (28.7)	-	-
May	584	347 (59.4)	223 (40.1)	14 (2.4)	-
June	465	206 (44.3)	200 (43.0)	49 (10.5)	10 (2.2)
July	422	184 (43.7)	149 (34.9)	68 (16.3)	21 (5.1)
August	539	289 (53.6)	169 (31.3)	61 (11.3)	20 (3.8)
September	569	322 (56.5)	182 (32.4)	48 (4.3)	17 (2.8)
October	257	92 (35.8)	135 (51.7)	30 (11.7)	-
November	127	40 (31.5)	75 (59.1)	12 (9.4)	-
December	42	14 (33.2)	28 (65.8)	-	-

In these months, ticks were not found in any geographical area
*- In parentheses is the partial share of ticks in percentages.

Species-specific seasonal prevalence of pasture ticks.

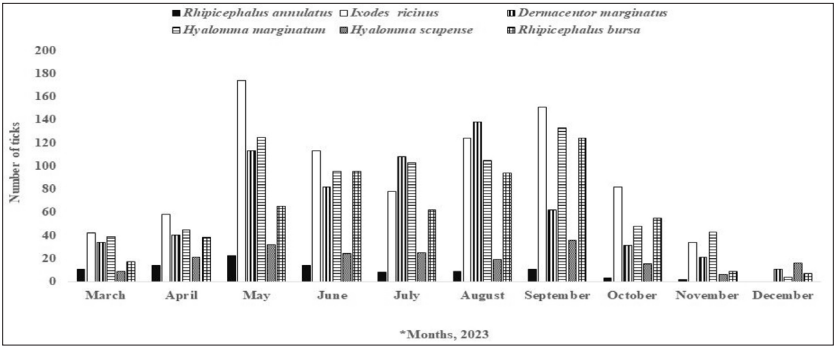

Figure 1. Quantitative distribution and species composition of ticks collected in the Berd locality of the Tavush Province from March to December 2023.
* No ticks were detected during the January-February observations.

Figure 1 shows the species-specific prevalence of pasture ticks collected from March to December 2023 in the Berd Region. As anticipated, tick numbers peaked from May to September, demonstrating a clear relationship between tick distribution and species type (Figure 1).

Brucellosis animal cases

According to current investigations, cases of brucellosis have been observed in the studied area since the 1950s. Interestingly, the foci of ignition are often new, but with small deviations from the old foci.

The rapid growth of population, particularly in areas with weak health systems, urbanization, globalization and inequalities within cities, climate change, and the changing nature of pathogen transmission between human and animal populations are an important cause of zoonoses [22]. Brucellosis is an endemic zoonotic disease leading to vast losses to the livestock industry and small-scale livestock holders [31]. The species of *Brucella melitensis, B. abortus*, and *B. suis* are the widespread causative agents of zoonosis inducing severe illness in humans [20, 41]. Zhang et al. 2019). The new species of *Brucella*: *B. microti, B. inopinata* and isolates from Australian rodents and amphibians, have also been described during the last decade [23]. *Brucella* spp. can survive for long periods for more than 2 years [29]. There are no vaccines for humans; on the other hand, conventional brucellosis vaccines for livestock do not show comprehensive protection [40]. Several risk factors such as animal sex and stock replacement were reported to be potential risk factors for brucellosis at the animal level [24]. Another association on brucellosis between the pet dogs and their owners was also found [32]. Soil was reported as a reservoir for the *Brucella* strain (*B. microti)* with high metabolic activity [37]. Interestingly, if distance near to water irrigation source, distance near to animal market, and animal density per village as well as manganese, lead, zinc, silt, clay, organic matter, and soluble salts were significantly associated with the prevalence of soil-borne *Brucella* spp., the distance from the main road and the soil pH, sand, moisture, nitrogen, phosphorus, nickel, cadmium, copper, chromium, iron, calcium, magnesium, sodium, and potassium were less associated with the pathogen [24].

Brucellosis has been registered on the territory of the Republic of Armenia since 1930 of the last century, but the mass spread of this infection began after the decentralization of collective and Soviet livestock farms with the redistribution of animals in private farmsteads and uncontrolled movement of livestock from one administrative-territorial unit to another. Over five years from 2008 to 2012, 10 out of 15 districts, subjected to epizootological examination, were

struck by brucellosis in cattle and small ruminants. At the same time, the share of this infection in comparison with other infectious diseases was 26.28%. According to the study, since 2010 the epizootic situation in brucellosis of cattle has sharply deteriorated. The number of animals, reacting to brucellosis, has increased. Nidus of infection of brucellosis of cattle, sheep, and horses in communities of Armavir and Aragatsotn areas of the Republic is open. In 2010 it was allocated 0.61, in 2011 – 0.22, and 2012 – 0.81% of animals responding to brucellosis. The infection rate for the same period increased by 32.8 times. Because of mass seromonitoring, carried out in the farms of the Kotaik region in 2015, 30% of the total number of examined cattle were revealed and subjected to forced slaughter. A high prevalence of brucellosis was also noted in sheep farms of the Republic. Only for the first 2008 year of the study, the incidence rate of sheep brucellosis exceeded that of cattle for all the years of the study to 0.93 %. The dynamic of incidence of sheep brucellosis increased about three times in 2009 compared with 2008. The most cases of the disease in Armenia were described for the regions of Ararat (53), followed by Kotayk (49), Armavir (38), Aragatsotn (36), Yerevan (28), Gegharkunik (26), Vayots Dzor (24), Syunik (8), and Lori (3) in the range of the "One Health Surveillance of *Brucellosis* in Armenia" initiated in 2016 (Asoyan et al. 2017). According to the authors, at the highest risk for brucellosis were males who worked with livestock, about 71% of patients had acute brucellosis with fever, arthralgia, and night sweating and 29 % suffered chronic brucellosis [2, 15]. About the Berd region of Tavush; as shown by current research data, outbreak foci are often new, but with small deviations from old foci of brucellosis. This, along with other encounters affecting the incidence of brucellosis, may also be due to the presence of pasture ticks. This hypothesis, although requiring in-depth molecular biology studies to prove the spread of the bacteria through ticks, suggests that there is a possibility of brucellosis outbreaks through ticks. These findings have significant implications for the development of zoonotic disease control strategies and the optimization of pasture allocation for livestock.

Conclusions

The microbiota of humans [7; 10-12; 18-19] animals [5-6; 8-9], and plants [10; 20] plays a crucial role for both the host organisms and their environment. Research into the bacteriome of the *R. microplus* cattle tick revealed geographical variations in bacterial accumulation within their microbiota [25]. Similar distinctions were observed among the tick species *R. annulatus*, *R. bursa, I. ricinus, D. marginatus, H. marginatum*, and *H. mcupense* prevalent in the Ta-

vush distinct, underscoring the importance of further microbiological analysis of tick microbiota from a biosecurity perspective.

In the Berd region, distinct ecological zones including Plains, Forest, Subalpine, and Alpine areas have been identified. The Plains zone spans elevations from 530 to 850 meters above sea level, the Forest zone ranges from 850 to 2000 meters, the Subalpine zone covers 2000 to 2200 meters, and the Alpine zone extends from 2200 to 2900 meters. These natural habitats offer conducive conditions for the proliferation of arthropods, particularly ticks in the wild. Research indicates that the prevalence and intensity of tick populations decrease with increased elevation. Notably, the degree of tick infestation varies significantly, ranging from a single tick to several hundred ticks on a single animal. The findings from this study are crucial for developing tick management strategies and for optimizing the allocation of grazing areas for livestock. Future research will focus on exploring the prevalence dynamics and genetic diversity of microorganisms within different tick species across these varied geographical zones.

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