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Research Article

Epidemiology of Tropical Theileriosis in the Cappadocia Region

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Abstract: A total of 554 cattle from the Cappadocia region were divided into 5 different groups according to vaccination and were examined for *Theileria annulata* by microscope and indirect fluorescence antibody test (IFAT). The sampled cattle were checked for tick infestation. The prevalence of *T. annulata* was 60.5% by microscopic examination and 67.5% by IFAT. According to the microscopic examination, the prevalence of *T. annulata* was significantly ($P < 0.001$) higher in the unvaccinated group. The seropositivity according to IFAT was significantly ($P < 0.001$) lower in the unvaccinated group. The morbidity was significantly ($P < 0.001$) higher in the unvaccinated, semi-grazed, pure breed, and >2 years age groups. The mortality was significantly higher in the unvaccinated ($P < 0.001$), semi-grazed ($P < 0.05$), pure breed ($P < 0.05$), and female ($P < 0.001$) groups. A total of 1585 tick specimens belonging to the genera *Hyalomma, Rhipicephalus, Dermacentor, Boophilus,* and *Ornithodorus* were detected in 21.48% of examined cattle. The developing forms of *T. annulata* were determined in the *H. anatolicum anatolicum* ticks microscopically. Out of 554 cattle, 156 (27.61%) were diagnosed with acute tropical theileriosis. Out of these 156 cattle, 86 (56.21%) died due to the disease.

Key Words: Cappadocia, epidemiology, tropical theileriosis

Kapadokya Bölgesinde Tropikal Theileriosis'in Epidemiyolojisi

Özet: Kapadokya yöresinde toplam 554 sığır aşılanma durumlarına göre 5 gruba ayrılmış ve bu hayvanlarda *Theileria annulata*'nın varlığı mikroskobik muayene ve indirekt floresan antikor testi (IFAT) ile araştırılmıştır. Örnek alınan sığırlar kene enfestasyonları yönünden kontrol edilmiştir. *Theileria annulata*'nın ortalama prevalansı mikroskobik muayenede % 60,5, IFAT'da ise % 67,5 bulunmuştur. Aşılanmamış sığırlarda mikroskobik muayene sonucu saptanan prevalans yüksek ($P < 0,001$) bulunurken seropozitifliğin oldukça düşük ($P < 0,001$) olduğu tespit edilmiştir. Seropozitiflik, aşısız grupta önemli derecede düşük bulunmuştur ($P < 0,001$). Morbitide, aşısız hayvanlarda ($P < 0,001$), zaman zaman meraya çıkan sığırlarda ($P < 0,001$), saf ırk ($P < 0,001$), ve > 2 yaş grubunda ($P < 0,001$) önemli derecede yüksek bulunmuştur. Mortalite aşısız ($P < 0,001$) zaman zaman meraya çıkan ($P < 0,05$), saf ırk ($P < 0,05$) ve dişilerde ($P < 0,001$) önemli derecede yüksek bulunmuştur. Muayene edilen sığırların % 21,48'inden *Hyalomma, Rhipicephalus, Dermacentor, Boophilus* ve *Ornithodorus* soylarına ait toplam 1585 kene toplanmıştır. *Theileria annulata*'nın gelişim formları *H. anatolicum anatolicum* kenelerinde mikroskobik olarak tespit edilmiştir. Toplam 554 sığırın 156'sında (% 27,61) akut tropikal theileriosis olgusu teşhis edilmiştir. Akut enfekte 156 sığırın 86'sı (% 56,21) enfeksiyona bağlı olarak ölmüştür.

Anahtar Sözcükler: Kapadokya, epidemiyoloji, tropikal theileriosis

Introduction

Ticks and tick-borne diseases (TBDs) cause major economic losses, and affect many domestic animals, mainly cattle and sheep, in tropical and subtropical regions (1).

Tropical theileriosis is a TBD caused by a protozoon called *Theileria annulata* transmitted by several tick species of the genus *Hyalomma* (2). The disease, threatening approximately 250 million cattle in the world, causes significant losses among the most productive cattle groups in the Mediterranean and

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subtropical countries in Europe, Africa, and Asia (3-5). Tropical theileriosis can be prevented by control or eradication of the vector ticks or by prophylactic immunization of target animals (6). The efficacy of acaricides against several *Hyalomma* spp. has been studied recently (7,8). Although some treatment studies have been reported in previously published articles (9,10), adequate treatment regimes to control or eradicate the vector ticks have not yet been critically evaluated (11).

Vaccination, tick control practices and grazing modalities are very important to establish the immunization status against tropical theileriosis in an endemic area (5,6,11,12). It has been reported that attenuated schizont vaccines have been used successfully for vaccination of susceptible cattle against tropical theileriosis in Israel, Iran, Morocco (11), and Tunisia (5).

The duration of immunity following sporozoite or schizont infections has not been determined yet, but does not appear to be lifetime. The attenuated, culture-derived anti-*Theileria* vaccine proved to be safe and effective in prevention of field theileriosis in large enzootic areas (11), even if revaccination might be required for cattle in herds with a low tick infection rate.

Tropical theileriosis is also the most important cattle disease in Turkey. Of the 10,761,000 cattle population, 6,544,000 (60%) pure and cross breed animals are at risk of the disease (13). It has been reported that imported non-immune cattle are highly susceptible to tropical theileriosis, with a mortality rate of more than 70% in pure breeds (particularly Friesians) and less than 45% in cross breeds; however, the mortality rate is approximately 15%-20% when the animal is exposed to the parasite at an early age, acquiring a preimmunity after recovery (13,14). It has been reported that approximately 150,000 susceptible cattle are vaccinated against tropical theileriosis each year in Turkey (15).

Malign *T. annulata* infections have been reported using traditional methods from all 7 geographical regions in Turkey (13,16), while benign *Theileria* parasites have been reported using molecular methods such as reverse line blotting (RLB) (17) and polymerase chain reaction (PCR) (18) from some parts of Turkey as well in other countries (19-21). Both field (13) and experimental studies (22) showed that the vector ticks of *T. annulata* are *H. a. anatolicum, H. a. excavatum, H. detritum detritum,* and *H. marginatum marginatum* in Turkey. In Cappadocia, which is one of the major parts of Central Anatolia, *T. annulata* and its vector ticks were reported (23).

The objective of this study was to evaluate epidemiological aspects and effectiveness of vaccination against *T. annulata* infection of cattle in Cappadocia.

Materials and Methods

Study area: The study was carried out in the Cappadocia area, a major part of Central Anatolia including the provinces of Nevşehir, Niğde, and Kayseri in Turkey (Figure). It includes the territory of 24 villages.

Management of herds: The herds from the selected villages are moved to the pastures in the daytime for grazing in the morning and are brought back to the village in the evening. The grazing season extends from March to December. A wide range of crops, including cereals, sugar beet, sunflower, vegetables, and animal fodder are grown in the pastures. The animals are subjected to indoor feeding during the winter months. The shelters where the cattle are kept during the indoor feeding time are constructed from sun-dried bricks and are characterized by poor hygienic conditions. In addition, sheep, goats, horses, and chickens are often kept in close contact with cattle.

Animal population: The district has approximately 16,000 cattle, mainly crossbreeds. Out of the 16,000 cattle, 554 (3%) were included in this study. Out of these 554 cattle, 339 are pure breed, 170 are crossbreed, and 45 are local breed; 69 are males and 485 are females; 135 are in the 0-1 year age range, 53 are in the 1-2 year range, and 366 are >2 years old. Three hundred forty-six (62%) of the sampled cattle were vaccinated against tropical theileriosis by attenuated schizont vaccine at a dose of 10^7. The vaccinated cattle (346) were divided into 4 groups as follows: vaccinated in 1999 (group I, 165 cattle), revaccinated in 2000 (group II, 104 cattle), and not revaccinated in 2000 (group III, 61 cattle) as subgroups of group I and the first time vaccinated in 2001 (group IV, 181 cattle). The earlier vaccination status of cattle in group I was not known and therefore group IV was used to monitor the status of first time vaccinated animals. All vaccinated and unvaccinated control cattle (group V, 208 cattle) were also divided into 2 subgroups as semi-grazed and zero-grazed. Tick control practices (TCPs) were applied to some of the herds using acaricides. It was observed that the avermectins were the

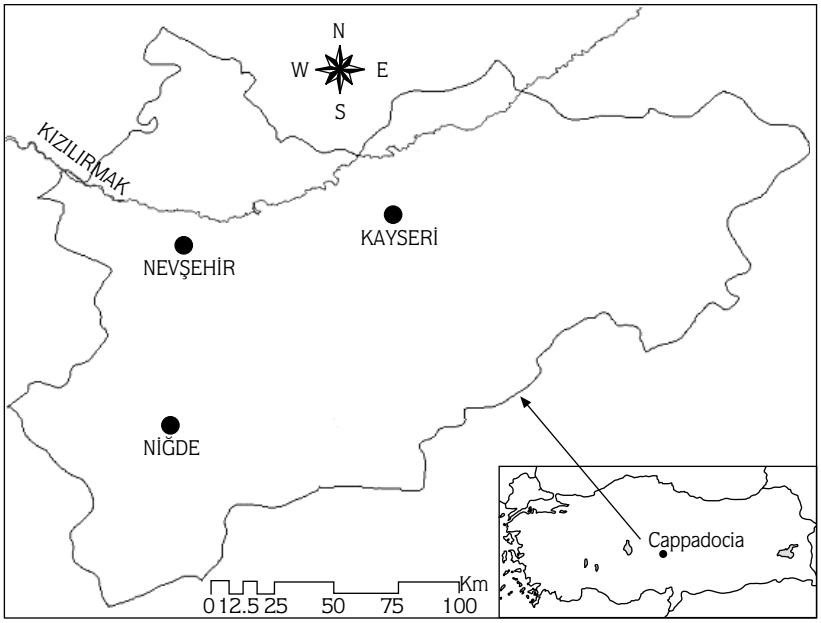

Figure. Map of the study area in the Cappadocia region.

most widely used on beef animals as injections while formamidins, pyrethroids, and organophosphates were applied to lactating dairy cattle as periodically body spray or pour-on during the tick infestation season.

Cattle sampling and data collection: Field work was conducted from April 1999 to November 2001. During the field studies, each village was visited several times. Particularly during the theileriosis season, some villages were visited more often to investigate *T. annulata* infections. Two field procedures (clinical examinations and questionnaire) were used in this study, providing clinical and anamnestic data during one herd visit. During the clinical inspection, individual animals were screened to estimate the prevalence of clinical theileriosis. Individual rectal temperature was recorded; measured temperature ≥ 39.5 °C was considered a febrile reaction. Vaginal mucosa and conjunctiva of the suffering cattle were inspected for petechial hemorrhages. The superficial lymph nodes (pre-scapular) were examined. Biopsy material was taken from the enlarged lymph nodes (≥++) to prepare smear slides. Blood was withdrawn from the jugular vein of each animal into vacutainers to separate serum samples. Blood with anticoagulant was also withdrawn to measure the level of packed cell volumes (PCVs). All smears were examined for schizont and piroplasm forms of *T. annulata*. At the same time, each animal was examined for tick infestations. The ticks found were collected from the infested cattle.

During the study period, the breeders were also interviewed. A questionnaire, similar to that described by Thrusfield (24), was used to collect information on cattle, management, environmental characteristics (wild life, climatic conditions) and risk factors for tick-borne diseases (TBDs). The cattle-based factors included age, sex, breed (indigenous, crossbreed, and imported cattle) and immunization status. Farm-based factors were TCPs, breeding practices, herd dynamics (births, deaths, sales, and purchases as well as their geographical origin), diagnosis and reporting of diseases, and the number of clinical cases and deaths due to TBDs recorded during the previous years.

Microscopic examination: Thin and thick peripheral blood smears and lymph node biopsy smears were stained with Giemsa (5% solution of Giemsa in buffer at pH 7.2) for 40 min and then examined at 1000 magnification.

Serological testing for *Theileria annulata* antibodies: The indirect fluorescent antibody test (IFAT) using both the schizont and piroplasm stages of *T. annulata* parasites as antigens was used to examine serum samples for the presence of the appropriate *T. annulata*-specific antibodies (25).

Tick infestation and the detection of *T. annulata* infection in *Hyalomma* species: The selected cattle were checked for tick infestation and the ticks found were removed from the perineum, tail base, udder, scrotum, and preputial flap. Identification was performed up to species level for *Hyalomma* spp. and up to genus level for other genera, following the Hoogstraal keys (26). Collected engorged nymphs of *Hyalomma* species were placed in an incubator at 28 °C and 85% relative humidity to allow them to molt into adults. Unfed adult *Hyalomma* ticks collected from the cattle were dissected and their salivary glands were stained with methyl green/pyronin as described by Walker et al. (27).

Statistical analysis: Chi-square ($\times^2$), Mann-Whitney, and Kruskal-Wallis tests were employed for statistical analysis of the results using SPSS 13.0.

Results

Clinical reactions: The observed clinical reactions of the exposed cattle with *T. annulata* in the Cappadocia region are summarized in Table 1.

Tick infestations: Tick infestations were detected in 119 of the 554 cattle (21.48%). A total of 1341 adults (707 males, 634 females) and 240 nymphs of ixodid ticks, and 4 adult argasid ticks were collected from the infested cattle. The adult ixodid ticks were identified as *H. m. marginatum* (n: 272), *H. d. detritum* (n: 181), *H. a. anatolicum* (n: 311), *H. a. excavatum* (n: 211), *Rhipicephalus bursa* (n: 34), *R. turanicus* (n: 36), *R. sanguineus* (n: 15), *Dermacentor niveus* (n: 16), and *Boophilus annulatus* (n: 265). The adult argasid ticks were identified as *Ornithodorus lahorensis*. The collected nymphs were identified as *H. a. anatolicum* (n: 116) and *B. annulatus* (n: 124).

***Theileria annulata* infection in *Hyalomma* species:** The developing forms of *T. annulata* were determined only in the *H. a. anatolicum* ticks with a prevalence value of 17.49%.

Microscopic and serological results, and morbidity/mortality: The microscopic and serological prevalence and morbidity/mortality are summarized in Table 2. The mean prevalence of *T. annulata* was 60.5% by microscopic examination and 67.5% by IFAT.

Discussion

The results of this study showed that the Cappadocia region is an endemic area for tropical theileriosis. This is consistent with the previous reports (13). Cappadocia has a very suitable climate and ecosystems for the development of ixodid ticks; thus, potential risk is constantly high for TBDs. This could be the explanation why Cappadocia is an endemic area for tropical theileriosis. In the present study, tick species obtained from the infested cattle were similar to those found in previous studies in Central Anatolia (13,23). Both field and experimental studies reported that those tick species transmit *Theileria* species (13,16,22). Theilerial masses were found in the salivary glands of unfed adult *H. a. anatolicum* ticks collected from the cattle during this study as described by Walker et al. (27). However, the prevalence of tick infections could be affected by some factors such as management of herds and the practice of keeping sheep and goats together with cattle in the same shelters and pastures. The results of the present study confirmed a previous published article (13).

There is clear evidence of differences in susceptibility of different cattle types and breeds to *T. annulata* infection. The prevalence of piroplasmic forms of *T. annulata* in cross and local breeds is higher than that in pure breeds in the present study. The probable reason for this was that all age groups of indigenous and cross breed cattle were permitted to be exposed to *Hyalomma* ticks throughout theileriosis season. Although pure breed animals were limited or not grazed during the study period, the observed high morbidity in pure breeds compared to cross+local breeds can be explained by environmental factors. It is known that pure breeds are more sensitive to the disease compared to cross+local breeds. This result is in agreement with previously published articles by Spickett et al. (28) and Sayin et al. (13). In parallel to tick infestation, the incidence of disease with severe clinical reactions and rapid decline in the mean PCV values increases in June and peaks in July, which is the known *Theileria* transmission season in the area. In this study, clinical symptoms observed in cattle with *T. annulata* were similar to those described previously (14).

Higher prevalence and lower seropositivity of *T. annulata* in the unvaccinated cattle group compared with the vaccinated cattle group can be explained by effects of vaccination. Previous studies reported that vaccination of

Table 1. Clinical reactions of the infected cattle with *T. annulata* in the Cappadocia region.

Groups	Body temperature (°C)**					PCV (%)					Enlargement of lymph nodes***				
	X ± Sx	Min.	Max.	$\times^2$	P	X ± Sx	Min.	Max.	$\times^2$	P	X ± Sx	Min.	Max.	$\times^2$	P
I	39.84 ± 0.67[a]	38.90	41.20	45.824*	< 0.001	25.50 ± 7.32[a]	12.00	34.00	36.883*	< 0.001	1.71 ± 0.83[a]	1	3	38.989*	< 0.001
II	39.50 ± 0.00[a]	39.50	39.50			29.00 ± 3.47[a]	26.00	34.00			1.00 ± 0.00[a]	1	1		
III	40.77 ± 0.76[b]	36.60	41.50			23.00 ± 8.10[a]	12.00	32.00			2.11 ± 0.78[b]	1	3		
IV	39.63 ± 0.18[a]	39.50	40.00			22.45 ± 6.90[a]	10.00	34.00			1.40 ± 0.82[a]	1	3		
V	40.38 ± 0.65[b]	39.00	41.50			16.44 ± 5.66[b]	8.00	28.00			2.51 ± 0.71[b]	1	3		
	X ± Sx	Min.	Max.	U	P	X ± Sx	Min.	Max.	U	P	X ± Sx	Min.	Max.	U	P
Dead	40.25 ± 0.66	39.00	41.50	2805.5	0.666	12.94 ± 3.08a	8.00	22.00	87.5	< 0.001	2.71 ± 0.59	1	3	965	< 0.001
Recovered	40.21 ± 0.71	38.90	41.50			25.00 ± 4.19b	12.00	34.00			1.69 ± 0.78	1	3		

I: vaccinated in 1999; II: revaccinated in 2000; III: not revaccinated in 2000; IV: first vaccinated in 2001; V: unvaccinated

*: Kruskal-Wallis test was performed.

**: Body temperature was taken every field visit.

***: The enlargement degree of lymph nodes was shown as 1:+, 2:++, 3:+++

[a, b]: Statistical differences between groups with different letters in the same column were significant.

Table 2. Epidemiologic aspects of tropical theileriosis in the Cappadocia region.

Variable	Number of cattle (%)	Positive cattle								Morbidity and Mortality							
		Microscopy (%)*	χ^2	P	df	IFAT (%)*	χ^2	P	df	Cases (%)	χ^2	P	df	Deaths (%)	χ^2	P	df
Groups																	
I	165 (29.8)	56 (33.9)[a]	23.5	< 0.001	4	140 (84.8)[a]	208.9	< 0.001	4	14 (8.5)[a]	157.2	< 0.001	4	3 (21.4)			
II	104 (63.0)	50 (48.1)[a]				94 (90.4)[a]				4 (3.8)[a]				0 (0.0)			
III	61(37.0)	26 (42.6)[a]				42 (68.9)[b]				9 (14.8)[a]				3 (33.3)			
IV	181(32.7)	81 (44.8)[a]				164 (90.6)[a]				20 (11.0)[a]				4 (20.0)			
V	208 (37.5)	122 (58.7)[b]				70 (33.7)c				106 (51.0)[b]				76 (71.7)			
Grazing																	
Semi-grazed	331 (59.7)	269 (81.3)[a]	148.8	< 0.001	1	262 (79.2)[a]	50.8	< 0.001	1	135 (40.8)[a]	71.3	< 0.001	1	81 (60.0)[a]	5.5	0.020	1
Zero grazed	223 (40.3)	66 (29.6)[b]				112 (50.2)[b]				18 (8.1)[b]				5 (27.8)[b]			
Vaccination																	
Vaccinated (I + IV)	346 (62.4)	213 (61.6)[a]	0.5	0.498	1	304 (87.9)[a]	174	< 0.001	1	47 (13.6)[a]	90.8	< 0.001	1	10 (21.3)[a]	31.6	< 0.001	1
Unvaccinated	208 (37.6)	122 (58.7)[a]				70 (33.7)[b]				106 (51.0)[b]				76 (71.7)[b]			
Breed																	
Pure	339 (61.2)	182 (53.7)[a]	16.8	< 0.001	1	220 (64.9)[a]	2.7	0.099	1	111 (32.7)[a]	11.5	< 0.001	1	69 (62.2)[a]	5	0.016	1
Cross + local	215 (38.8)	153 (71.2)[b]				154 (71.6)[a]				42 (19.5)[b]				17**(40.5)[b]			
Age (Years)																	
0-1	135 (24.4)	56 (41.5)[a]	28.2	< 0.001	2	72 (53.3)[a]	17.8	< 0.001	2	19 (14.1)[a]	18.1	< 0.001	2	8 (42.1)[a]	2.6	0.272	2
1-2	53 (9.6)	39 (66.1)[b]				42 (79.2)[b]				13 (24.5)[b]				6 (46.2)[a]			
> 2	366 (66.0)	240 (65.6)[b]				260 (71.0)[b]				121 (33.1)[b]				72 (59.5)[a]			
Sex																	
Male	69 (12.4)	32 (46.4)[a]	6.5	0.010	1	50 (72.5)[a]	0.6	0.423	1	13 (18.8)[a]	2.5	0.110	2	1 (7.7)[a]	11.5	< 0.001	1
Female	485 (87.6)	303 (62.5)[b]				324 (66.8)[a]				140 (28.9)[a]				85 (60.8)[b]			
Total	554 (100)	335 (60.5)				374 (67.5)				153 (27.6)				86 (56.2)			

I: vaccinated in 1999; II: revaccinated in 2000; III: not revaccinated in 2000; IV: first vaccinated in 2001; V: unvaccinated

[a, b, c]: Statistical differences between groups with different letters in the same column were significant.

* Kappa: 0.521, P < 0.001

**: No cattle in local breed

target animals against *T. annulata* before the disease season each year is effective in preventing tropical theileriosis in endemic areas (4-6,11,12,14,15). Moreover, high morbidity and mortality rates in the unvaccinated cattle group compared to the vaccinated cattle group were expected and consistent with the results of previous studies (4,11,14). On the other hand, a significant number of animals were infected with disease in the vaccinated cattle group. This could be related to the different strains of *T. annulata* as stated previously (13). It may also be associated with the practice of revaccination or no revaccination and the manipulation mistakes of the vaccination in the field.

The higher prevalence of *T. annulata* resulting in high morbidity and mortality in the semi-grazed cattle group compared with the zero-grazed cattle group can be explained by the easier natural tick infestation in those cattle during the theileriosis season in an endemic area.

The higher prevalence in the ≥1 year old group compared with the <1 year old group and the higher morbidity in the >2 years age group compared with the others can be explained by the fact that young animals were kept indoors. Therefore, the risk of natural tick infestation was reduced. For the same reason, it could be explained that females had higher prevalence than males, since males are mostly kept indoors or are limited in grazing. Indoor feeding of males is a traditional management practice in the region.

Consequently, the results of this study also showed that there is a serious immunization problem of susceptible cattle against tropical theileriosis in the Cappadocia region as in the rest of Turkey. Vaccination is not sufficient in itself to limit the infection and the economic losses, even if it limits the clinical cases. The resolution of this problem may be possible using new approaches such as the establishment of a new subunit vaccine and a defined vaccination strategy in the region as well as in the rest of Turkey. The vaccinated cattle must be grazed and challenged by tick infestation to establish a strong immunity against tropical theileriosis during the season. The immunized cattle in this way (vaccination and grazing) do not need revaccination against the diseases in the next theileriosis season. In addition, revaccination is very important before the next disease season in each year for immunized cattle that have been kept inside (zero grazed) during the disease season as well as for susceptible cattle.

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