



Studies on the Epidemiology of Tropical Theileriosis (*Theileria annulata* Infection) in Cattle in Central Anatolia, Turkey

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ABSTRACT

An epidemiological survey for *Theileria annulata* infection was conducted in 12 selected villages around Ankara in Central Anatolia, Turkey, during the period April 1990 to January 1993. During the survey, 198 cattle of 30 local breeds, 84 Holstein-Friesian × local breeds and 84 Holstein-Friesian breed were examined for antibodies to *T. annulata* and the presence of the vector ticks. Four species of *Hyalomma* ticks were identified: *Hyalomma anatolicum anatolicum*, *Hyalomma anatolicum excavatum*, *Hyalomma detritum* and *Hyalomma marginatum marginatum*. Salivary gland staining indicated that infected adult ticks of all four species were present and, therefore, were implicated in the transmission of tropical theileriosis in the field. Generally, the *Hyalomma* infestation rate was low, with the heaviest infestations occurring on the older animals. Young adults and calves had very low infestation rates. Most ticks seen on cattle were adults, very few nymphs were found. The blood smear and serological examination of the 198 cattle conducted in March, before the start of the first disease season, showed that the prevalence of piroplasmosis was 11.1% (22 out of 198) and the seroprevalence of *T. annulata* was 10.6% (21 out of 198). Forty-three animals were then excluded from the study because they were seropositive and/or harboured piroplasms. Ninety-two seronegative animals showed piroplasmosis (92 out of 155) and 34 seronegative animals became seropositive for *T. annulata* (34 out of 155) during the three disease seasons. One animal became clinically ill with tropical theileriosis and required treatment. The incidence of cattle showing piroplasmosis and disease in the total study sample was 50.7% and 0.5% per disease season, respectively. The seroconversion rate of new infection with *T. annulata* in the total study was 14.3% per animal season. The number of cattle showing piroplasmosis was much greater than the number of seropositive cattle, which may indicate the presence of another species of *Theileria*. The two different management systems encountered in the study were considered to have influenced the tick infestation levels.

Keywords: breed, cattle, diagnosis, epidemiology, *Hyalomma*, infestation rate, prevalence, serology, *Theileria annulata*

Abbreviations: GUTS, ground-up tick supernatants; HPF, high-power field; IFAT, indirect fluorescent antibody test; PBMC, peripheral blood mononuclear cell

INTRODUCTION

Tropical theileriosis, caused by the protozoan parasite *Theileria annulata*, is the most important cattle disease in Turkey and occurs throughout the country. *T. annulata* has been reported from all seven geographical regions in Turkey, namely Central Anatolia (Göksu, 1959, 1970; Özcan, 1961; Ünsüren *et al.*, 1988), Eastern Anatolia (Dumanli and Özer, 1987; Göksu, 1970), South Eastern Anatolia (Mimioglu *et al.*, 1971; Göksu, 1970), Black Sea Coast (Göksu, 1970; Mimioglu, 1955; Acici, 1983), Marmara (Göksu, 1970; Tüzer, 1981), Aegean Coast (Erkut, 1967; Göksu, 1970) and the Mediterranean Coast (Göksu, 1970).

The Central Anatolian plain is an endemic area for tropical theileriosis (Göksu, 1959; Özcan, 1961; Ünsüren, 1976). The disease was described by Göksu (1959) and Ünsüren (1976) and an epidemic of the disease in South Eastern Anatolia, which was reported by Mimioglu and colleagues (1971), resulted in many fatalities among exotic Friesian/Holstein cattle and their crosses. The parasite has also been reported in the water buffalo (*Bubalus bubalis*) in Turkey (Tasci, 1984).

Previous studies have dealt with infections diagnosed by microscopic examination of blood smears, clinical signs and specific chemotherapeutic treatment of the disease, but there has been no serological study to evaluate the distribution, incidence and prevalence of *T. annulata* in Turkey.

Tropical theileriosis occurs over a broad area from North Africa to China, causing death and production losses. The disease is controlled in several countries by immunizing cattle with live, attenuated *T. annulata*-infected cell culture vaccines (Pipano, 1977; Fesharki, 1988; Stepanova and Zablotskii, 1989). A live, attenuated cell culture vaccine is produced in Turkey and is targeted towards protecting the large number of highly susceptible exotic cattle imported from Europe and North America (Onar, 1989). However, many basic epidemiological questions about the incidence of the disease and its effects on production losses remain unanswered.

The antitheilerial drug buparvaquone has been used effectively in the treatment of tropical theileriosis in the field (Ünsüren *et al.*, 1988). Despite these efforts to keep the disease under control, the incidence of tropical theileriosis in Turkey is increasing. The inability to control the disease by conventional methods is related to the lack of epidemiological knowledge. At present, the vaccine is being under-used and is not properly targeted at those disease areas where it is most needed. Studies on disease incidence and prevalence would provide the information required, thereby permitting the most efficient use of the available vaccine and of complementary control measures.

The epidemiology of East Coast fever, caused by the closely related parasite *Theileria parva*, has been studied more extensively (Young, 1981; Moll *et al.*, 1986). Field and laboratory data have been used to construct a mathematical model of the life cycle of the vector of East Coast fever, *Rhipicephalus appendiculatus* (King *et al.*, 1988). More recently, a similar study was carried out on tropical theileriosis in an endemic area of Morocco (Flach and Ouhelli, 1992; Flach *et al.*, 1995).

Several species of *Hyalomma* ticks transmit *T. annulata* (Robinson, 1982) but, in the countries of North Africa, tropical theileriosis is transmitted naturally by only one species, *Hyalomma detritum* (= *H. mauritanicum*) (Sergent *et al.*, 1945; Flach and

Ouhelli, 1992). This two-host tick feeds on cattle both at the larval and nymphal stages (year 1) and at the adult stage (year 2) (Hoogstraal, 1956). In North Africa, tropical theileriosis usually occurs between May and September and closely follows the abundance of adult *H. detritum*, which occurs between April and August (Sergent *et al.*, 1945).

In Israel, tropical theileriosis is also confined to those regions of the country where the two-host tick *H. detritum* occurs (Pipano, 1976). Three-host ticks, such as *H. anatolicum excavatum*, are capable of transmitting *T. annulata* under experimental conditions, but no field outbreaks have been associated with this species. Immature stages of *H. a. excavatum* are not found on cattle under natural conditions in Israel (Samish and Pipano, 1983; Pipano, 1989). In the countries of the former USSR, tropical theileriosis is transmitted by *H. a. anatolicum*, *H. detritum*, *H. plumbeum* and *H. scupense* (Stepanova and Zablotskii, 1989).

In Turkey, the common *Hyalomma* ticks are *H. detritum*, *H. a. anatolicum*, *H. a. excavatum* and *H. m. marginatum* (Mimioglu, 1954; Kurtpinar, 1954; Hoffman *et al.*, 1971). *Hyalomma detritum* acts on cattle as a two-host tick, *H. a. anatolicum*, *H. a. excavatum* and *H. m. marginatum* as three-host ticks (Kurtpinar, 1954; Merdivenci, 1969). Cases of tropical theileriosis occur from May until September, with the highest number occurring in July, in parallel with the increase of adult *Hyalomma* ticks between these months (Göksu, 1959; Özcan, 1961). However, the *Hyalomma* species that transmit the disease in field are not yet known. The objective of this serological study was to evaluate the distribution, incidence and prevalence of *T. annulata* in Central Anatolia and to investigate the tick transmission patterns.

MATERIALS AND METHODS

Field study area

The country of Turkey comprises approximately 779 450 km² and is situated between 36° and 42° N and 26° and 45° E. There are seven geographical regions (Figure 1), one of which is Central Anatolia.

Central Anatolia is an inland area of approximately 151 275 km² and consists of mountainous plateaux and valley plains. It is separated from coastal areas by high and long mountain ranges. The altitudes of the study sites ranged from 780 to 1100 m. The climate of Central Anatolia is typical of an inland subtropical area. The temperature varies widely, with cold winters, hot summers, and cool wet springs and autumns separating these two extremes. The rivers Kizilirmak, Yesilirmak, Seyhan and Sakarya, including two small dams, bisect the region. There are also two small lakes plus irrigated pastures agricultural lands and woodlands. There is mixed vegetation, which consists chiefly of species of *Artemesia*, *Thymus*, *Astragalus*, *Tris*, *Nipella*, *Crupina*, *Ziziphora*, *Jasminus*, *Clemastis*, *Crocus*, *Ornithopalum*, *Frankemia*, *Allium* and *Tragacanth*.

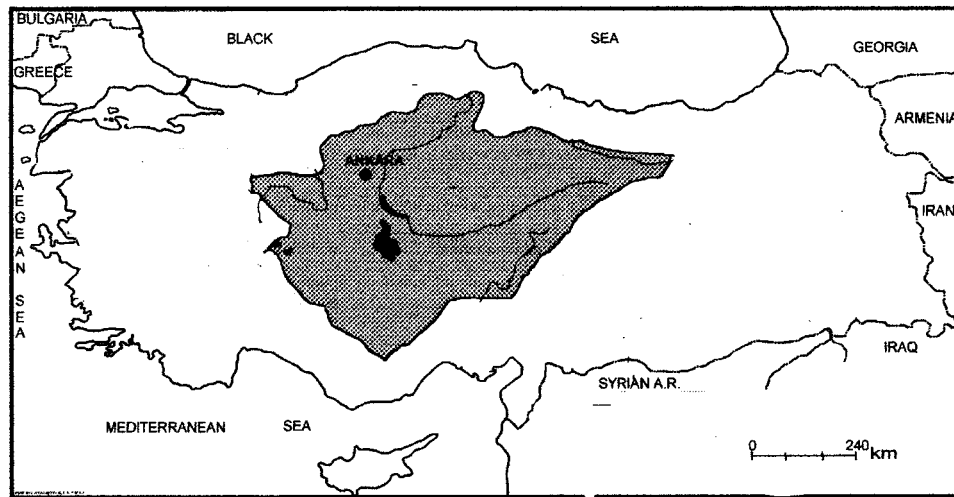


Figure 1. Map of Turkey. The Central Anatolia region is represented by the shaded area

The nature of the villages

The epidemiological study was carried out from April 1990 to January 1993 in 12 villages around Ankara County at approximately 40°N, 32° E (Figure 2). Following consultation with the Ministry of Agriculture Regional Office in Ankara, sites were selected from a list of villages where, on the basis of blood smear examinations, *T. annulata* infections were understood to occur and where the owners and veterinarians were willing to cooperate. The 12 selected villages (Eryaman, Fethiye, Bitik, Taspınar, Kizilca, Esenboga, Ogulbey, Karaoglan, Kayadibi, Yaglipinar, Karacahasan and Hasanoglan) were within 15–100 km of Ankara, in different directions from the city. These villages are typical of the area, and lie at altitudes between 780 and 1100 m, with populations between 210 and 2100. A wide range of crops, including cereals, sugar beet, vegetables and animal fodder, is grown and each village has a common cattle herd and common pasture or grassland, where the herd is grazed. The number of cattle in each herd varies between 120 and 400. Most of the herds are composed of about 50% crossbreeds, 30% local breeds and 20% European cattle (Holstein). In 10 of the villages, the herds are moved to the pasture in the early morning, after milking. They graze there all day before being brought back to the village in mid-afternoon for milking. In two villages, Kizilca and Hasanoglan, a different management system is employed, the cattle being fed indoors. The grazing season extends from March to December. During the winter months, all the cattle are fed indoors. The barns where the cattle are housed during the indoor feeding season are constructed of sun-dried bricks and are not usually hygienic. Cattle are the main source of milk and cash sales. The number of cattle each farmer possesses varies between 1 and 15. In addition, the breeders keep sheep, goats, horses and chickens. In the villages selected for the study, the cattle were neither vaccinated against tropical theileriosis nor treated regularly with acaricides.

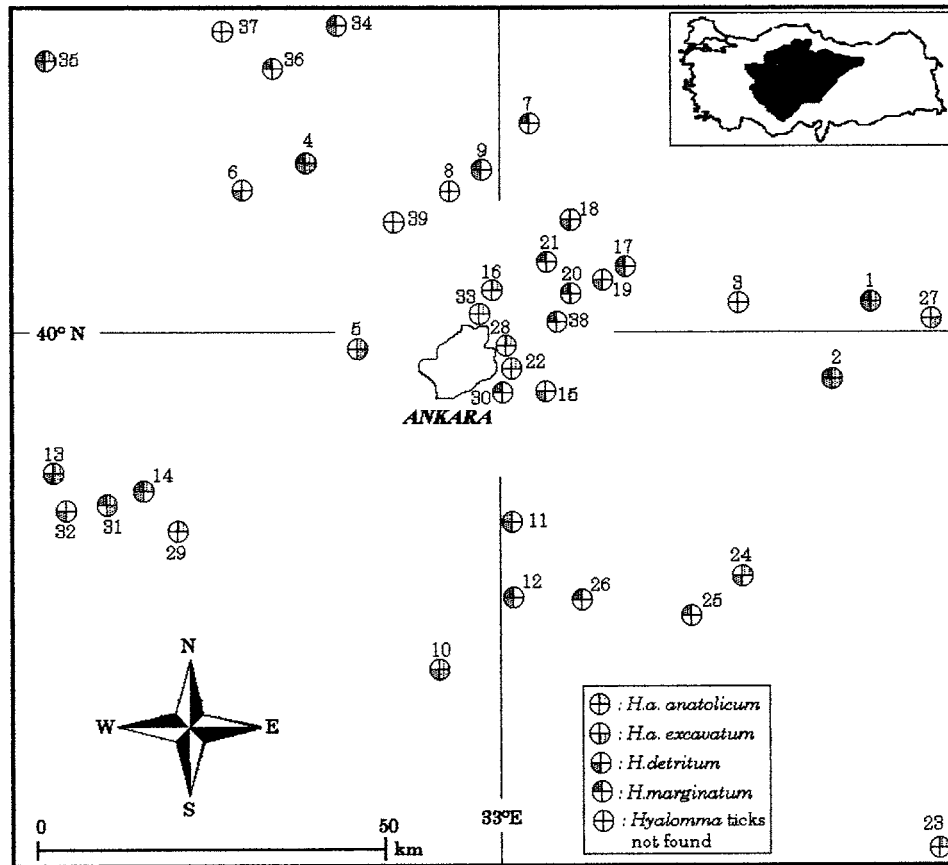


Figure 2. The villages where the epidemiological study was carried out: 1, Kayadibi; 2, Karacahasan; 3, Hasanoglan; 4, Bitik; 5, Eryaman; 6, Fethiye; 7, Taspınar; 8, Kızılca; 9, Esenboga; 10, Yaglipınar; 11, Karaoglan; 12, Ogulbey. Villages 13 to 39 were places where ticks were collected but which were not otherwise part of the epidemiological survey

The structure of the herds

The sample for the epidemiological study was drawn from the animals examined on the first visit to each of the 12 villages. A total of 107 cattle were selected in March 1990. In March 1991 and 1992, additional matched cattle, 25 and 23 respectively, were introduced to replace the cattle sold during the successive previous disease seasons, a disease season being defined as the period from April to October. The cattle were all 1 year old or older, and had usually grazed on the pastures for at least one disease season. In addition, 43 calves, aged between 4 and 6 months, and born after the previous disease season, were also introduced in March 1991. Thus, the total number of animals in the sample was 198, with each individual village having between 12 and 25 (mean 16.5) trial animals. Each animal was given a uniquely numbered ear tag and

the breed and age category were recorded. The cattle in the study comprised 30 local breeds, 84 Friesian-Holstein $\times$ local breed crosses and 84 Friesian-Holsteins.

Visits to the villages and sampling of the cattle

Each village was visited once a month from April 1990 to January 1993. The breeders were questioned about births, deaths, sales, and other losses among the sample population. In addition, they were asked about the use of acaricides, vaccination and the occurrence of tropical theileriosis and other diseases. The mouth, conjunctival mucous membranes and prescapular lymph nodes of any animal in the sample suspected of suffering from tropical theileriosis were examined clinically and biopsies were taken from enlarged nodes. Blood was withdrawn from the jugular vein of each animal into plain vacutainers on each visit and serum was separated from the blood. Thin and thick blood smears were made from blood collected from the tip of the tail. Thin blood smears were fixed for 2 min in methanol within 24 h of collection. Both thick and thin smears were stained with Giemsa stain for 40 min (5% solution of Giemsa stain in buffer at pH 7.2). The number of piroplasms in 200 high-power fields at $\times 1000$ magnification were recorded and expressed as the number per 200 fields (200 HPF). Similarly, lymph node biopsy smears were stained with Giemsa stain and examined for schizonts. Each serum sample was aliquoted into 1.5 ml microtubes and stored at -20°C until tested.

During each visit, the 107 seronegative non-carrier animals were examined for *Hyalomma* ticks. As part of a separate survey on tick populations, 175 animals (125 cattle plus 50 calves) in another 27 villages were also randomly checked for tick infestation. Adult *Hyalomma* ticks were removed from the perineum, tail base, udder, scrotum, and preputial flap. Immature ticks were also collected, placed in an incubator maintained at 28°C and 85% relative humidity and allowed to moult into adults. When engorged nymphs of *Hyalomma* species were found on the animals, they were allowed to moult into adults, when species identification took place. Unfed adult *H. a. excavatum* were also found in the cracks and crevices in the walls of cattle barns. Ticks were also collected from the cattle and barns in the other 27 villages within Ankara County (Figure 2). The genera and species of adult *Hyalomma* ticks were identified using the keys of Hoogstral (1956), Hoogstral and Kaiser (1959), Kaiser and Hoogstral (1964), Kurtpinar (1954) and Pomerantzev (1959). Immature ticks were identified to species level after moulting to adults. Unfed adult ticks collected from the animals were dissected without pre-feeding and their salivary glands were stained with methyl-green/pyronin as described by Walker and colleagues (1979) and Irvin and colleagues (1981). Two ground-up tick supernatants (GUTS) were prepared, one from 4 unfed *H. m. marginatum* ticks collected from a bovid in Kalecik, and the second from 17 unfed adult *H. a. excavatum* ticks which had moulted from engorged nymphs collected from cattle in Anayurt. Both these GUTS were used to infect separate aliquots of sterile peripheral blood mononuclear cells obtained from a susceptible calf, as described by Brown (1987).

Serological testing for T. annulata antibodies

The indirect fluorescent antibody test (IFAT) (Burrige and Kimber, 1972; Goddeeris *et al.*, 1982), using both the schizont and piroplasm stages of the *T. annulata* parasite as antigens, was used to examine serum samples for the presence of the appropriate *T. annulata*-specific antibodies. One hundred and ninety-eight cattle in the 12 selected villages were tested in 1990, before the start of the disease season.

Questionnaire

A questionnaire, similar to that described by Thrusfield (1986), was designed for veterinary practitioners and the Veterinary Faculty clinics in order to obtain information on the herd, farm and village structures, numbers of indigenous and European cattle, the situation with respect to vaccination, cases of theileriosis, methods of treatment, deaths, etc.

Data handling

Data were stored and analysed on a microcomputer using a database and spreadsheet. Tick infestation was illustrated using median, minimum and maximum values. Incidence rates were calculated as the number of new cases per animal month during the three disease seasons, with April to October defined as one disease season, multiplied by the number of months in the season to give a value per animal season (Thrusfield, 1986).

The mean monthly temperatures and relative humidity from 1990 to 1992 were obtained from the Ankara meteorological station and correlated with tick development. The mean maximum daily temperatures varied between 5°C and 6.5°C in winter, 15.3°C and 19.1°C in spring, 26.2°C and 28.8°C in summer, and 11.3°C and 21.8°C in autumn. The relative humidity varied between 70% and 76% in winter, 63% and 76% in spring, 52% and 60% in summer, and 54% and 72% in autumn.

RESULTS

Infestation with tick vectors

A total of 267 adult *Hyalomma* ticks were collected from 10 of the selected 12 villages between March 1990 and January 1993. The species and numbers are recorded in Table I. During the study period, 39 (25%) of the 155 animals became infested with *Hyalomma* ticks at least once. The number of ticks found on each animal varied between 1 and 10. Thirty-five (89.5%) adult cattle out of the 39 infested animals showed piroplasms. The number of piroplasms in the infected cattle varied between one and four in 200 HPF. While *H. a. anatolicum*, *H. a. excavatum* and *H. detritum*

TABLE I
Species and *Theileria* infection of *Hyalomma* ticks collected from the study sample

Ticks	Collected		Dissected	Infected		Mean infected acini per tick
	(n)	(%)	(n)	(n)	(%)	
<i>Hyalomma a. anatolicum</i>	27	10.11	12	1	8.33	1.0
<i>Hyalomma a. excavatum</i>	66	24.72	35	1	2.86	1.0
<i>Hyalomma detritum</i>	113	42.32	60	3	5.0	7.6
<i>Hyalomma m. marginatum</i>	61	22.85	45	0	0.0	0.0
Total	267	100	152	5	3.29	

n = number of ticks

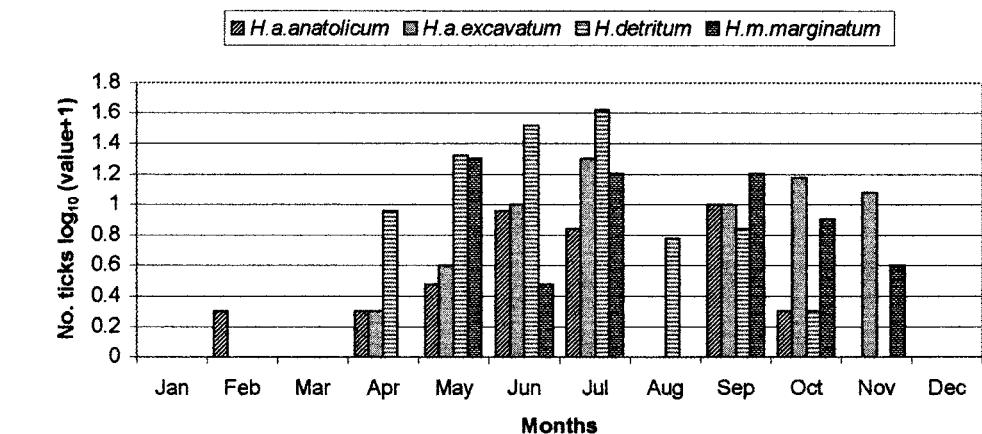
began to appear on the animals in the middle of April, *H. m. marginatum* was first seen at the beginning of May. Tick numbers remained low until May, rising to a peak in July, after which there was a sharp decline until the beginning of August when, apart from a few *H. detritum*, they virtually disappeared. A number of adults of the four species reappeared during September, October and early November (Figure 3a). Overall, four times as many male ticks were collected as females (213 vs 54).

All the adult cattle and calves in the study sample were free of immature *Hyalomma* ticks during all the visits to the 12 villages. However, in the villages not included in study site, larvae of *Hyalomma* species appeared on cattle in the first half of August and nymphs were collected from cattle between August and October, with a peak in September.

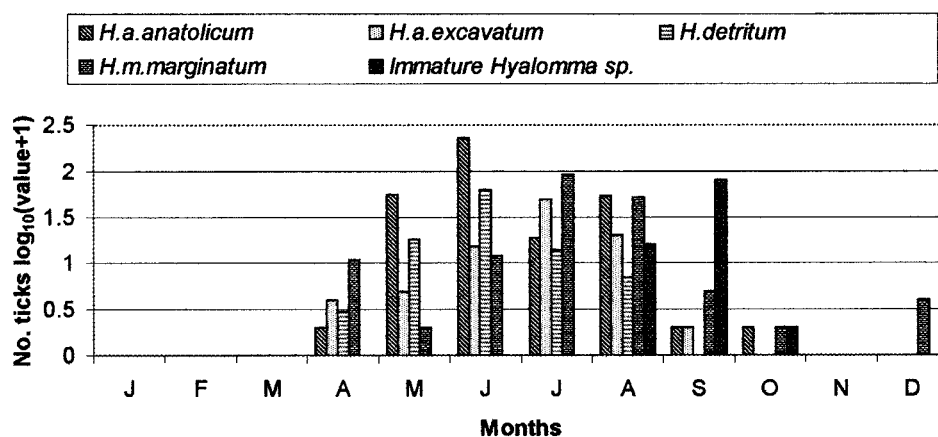
Fifty-seven per cent (152 of 267) of the adult *Hyalomma* ticks collected from the cattle in 10 of the villages during the study period were dissected and theilerial masses were found in five ticks (3.3%). The infection rates and mean infected acini per tick are recorded in Table I.

An additional 716 adult *Hyalomma* ticks were collected from 71 (62 adult cattle, 9 calves) of 175 animals (125 adult cattle, 50 calves) in the other 27 villages during the survey period. The numbers of ticks found on each infested adult animal and calf varied between 1 and 15, and between 1 and 3, respectively. The details are shown in Table II and Figure 3b.

A total of 217 adult *Hyalomma* ticks were collected from cracks and crevices in the walls of barns in two villages (Sarioba and Gures) between October and April. These comprised 5 *H. a. anatolicum* and 212 *H. a. excavatum*. *Hyalomma* nymphs were also found in the cracks and crevices in the walls of barns from the beginning of October to the end of November. Of the 94 *Hyalomma* nymphs removed from cattle in September, 25 were identified, after moulting, as *H. a. anatolicum* or *H. a. excavatum*. One *Hyalomma* larva collected from a cow and 10 live *Hyalomma* nymphs that were found in cracks in the wall of a barn did not moult into adults.



a



b

Figure 3. (a) Monthly distribution of adult *Hyalomma* ticks collected in the study samples during the period between March 1990 and September 1992. (b) Monthly distribution of *Hyalomma* ticks collected from cattle in the villages not included in the main survey

Among the unfed adults, 565 out of the 716 *Hyalomma* ticks were dissected. The tick species and their infection rates are recorded in Table II.

One mononuclear cell line infected with *T. annulata* was established from the infection of sterile PBMC and GUTS prepared from four unfed *H. m. marginatum* ticks collected from a bovid in Kalecik. The second GUTS, prepared from 17 unfed adult *H. a. excavatum* ticks, moulted from engorged nymphs collected from cattle in Anayurt, failed to establish an infection.

TABLE II

Species of *Theileria* and infection in *Hyalomma* ticks collected from the 27 villages not included in the main survey

Ticks	Collected		Dissected	Infected		Mean infected acini per tick
	(n)	(%)	(n)	(n)	(%)	
<i>Hyalomma a. anatolicum</i>	356	49.72	149	3	2.0	14.6
<i>Hyalomma a. excavatum</i>	89	12.43	132	1	0.8	3.0
<i>Hyalomma detritum</i>	99	13.82	102	5	5.0	31.6
<i>Hyalomma m. marginatum</i>	172	24.02	182	1	0.5	43.0
Total	716	100	565	10	1.7	

n = number of ticks

Theileria annulata infection

Of the 195 cattle sampled before the beginning of the first disease season, 21 (10.6%) were seropositive for *T. annulata* and 22 (11.1%) showed piroplasms and so were excluded from further study. Ninety-two of the 155 animals studied further in the 12 villages became carriers of *Theileria* piroplasms during the three disease seasons 1990, 1991 and 1992. Piroplasms were first seen in April, with higher occurrences in May and September (8 in April, 14 in May, 14 in June, 20 in July, 17 in August, 12 in September and 7 in October). In 57 cattle, only one piroplasm was seen in 200 HPF. The number of piroplasms varied between 2 and 1000 in the remaining 35 cattle. One animal became clinically ill with tropical theileriosis and showed schizonts. It was treated with buparvaquone (Butalex, Coopers Pitman-Moore). The incidence rates of piroplasm carriers and disease in the total study sample were 0.507 and 0.007 per animal season, respectively. None of the initial *Theileria* carriers developed clinical disease or showed any evidence of superinfection. The synchronization between the monthly prevalence of piroplasm carriers and tick infestation can be seen clearly in Figure 4.

Thirty-four animals in the 12 villages became seropositive for *T. annulata* against the 92 that became piroplasm carriers during the three disease seasons; 7 of the 34 appeared in May, the others in different months during the three years. Seropositive cattle showed high antibody titres against both piroplasm and schizont antigens of *T. annulata*. The incidence of seroconversion for new infections with *T. annulata* in the total study sample was 0.143 per animal season. The period during which the animals remained seropositive varied between 2 and 28 months. The number of animals that were seropositive for more than 3 months was 11, that is 32.3% of the seropositive animals.

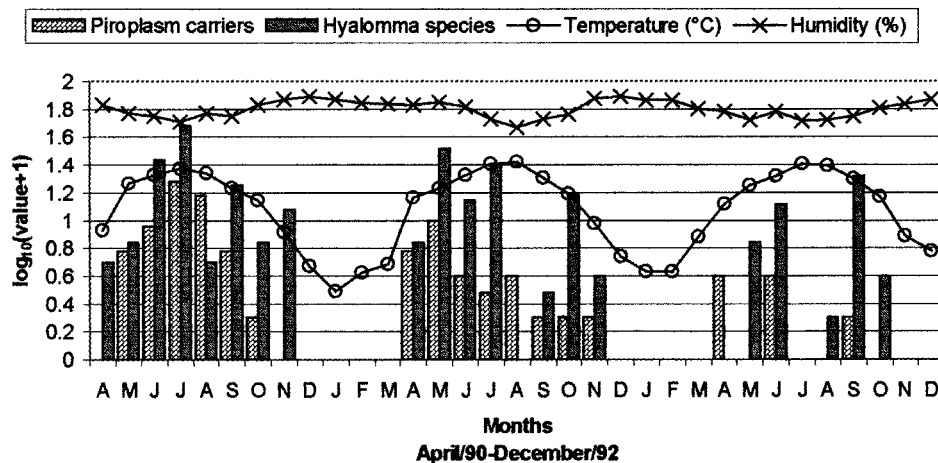


Figure 4. Synchronization between the monthly occurrence of piroplasm carriers and tick infestations in the study samples

Effect of age

Adult cattle were more heavily infested with adult *Hyalomma* ticks than calves. However, either most or all of the animals were free of adult ticks at every visit. As shown in Table III, the median total number of adult *Hyalomma* spp. collected from adult cattle was 8.5 (interquartile range, 6–57), while only one calf carried three ticks. Although all the adult cattle and the calves were free of immature ticks at all 12 visits to the 12 study villages (Table III), the adult cattle and calves in the other villages had approximately the same degree of infestation with immature ticks.

Before the disease seasons, 1 (2.3%) and 3 (7%) of 43 calves born after the previous September were seropositive for *T. annulata* and parasitaemic for *Theileria* species, respectively, whereas 20 (13%) of 155 adult cattle were seropositive for *T. annulata* and 19 (12%) carried piroplasms. The number of infections and clinical cases in each age group are given in Table IV.

Because new infections could only be detected in non-carriers of piroplasms, incidence rates were calculated only for these animals. Approximately 15.3% of susceptible calves became piroplasm carriers but none of them showed clinical reactions. Of the naive adult non-carriers, approximately 74% became piroplasm carriers and one showed a clinical reaction.

TABLE III
Tick infestation in the two groups of villages in the study

	Two zero-grazed management systems	Ten standard management systems
Total <i>Hyalomma</i> species		
Adults		
Median	0.0	8.5
IQR	–	6–57
Immature		
Median	0.0	0.0
Total <i>H. a. anatolicum</i>		
Median	0.0	0.50
IQR	–	0–4
Total <i>H. a. excavatum</i>		
Median	0.0	2
IQR	–	0–10
Total <i>H. detritum</i>		
Median	0.0	3
IQR	–	0–14
Total <i>H. m. marginatum</i>		
Median	0.0	1
IQR	0.0	0–11

IQR, interquartile range

TABLE IV
Incidence of detection of piroplasms and disease: seroincidence rates of *T. annulata* infection

Age ^a	Animal season at risk ^b	No. with new infection	Incidence rate	No. with disease	Incidence rate
1 ^c	52.57	6	0.114	0	0.0
2 ^c	128.85	86	0.667	1	0.007
1 ^d	54.57	2	0.036	0	0.0
2 ^d	183.00	32	0.174	0	0.0

^a1, calves born since end of the previous September; 2, adult cattle

^bNo. of animal months at risk (April–October)/no. of months

^cIncidence rates of piroplasm detection

^dSeroconversion incidence rates of *T. annulata* infection

Effects of breed

During the study, 6 (50%) of 12 cattle of the local breed, 14 (20.9%) of 67 crossbred cattle and 19 (25%) of 76 Holsteins became infested with *Hyalomma* ticks (Table V). The numbers of ticks on the animals varied between 1 and 10, with local breeds being more heavily infested than either crossbreds or Holsteins. Five of the six infested cattle of local breeds carried eight or more ticks.

Theilerial piroplasms were detected in 8 (66.6%) out of 12 cattle of local breeds, 44 (65.6%) out of 67 crossbred cattle and 40 (52.6%) out of 76 Holstein cattle. Two seroconverted animals (16.6%) were found among the cattle of local breeds, 13 (19.4%) in the crossbreds and 19 (25%) in the Holsteins. The incidence rate of piroplasm detection, seroconversion for *T. annulata* and percentage tick infestation are also recorded in Table V. The differences between the infection rates in the three categories were significant. Tropical theileriosis occurred only once, in one of the 76 Holstein cattle.

TABLE V

Detection of *Theileria* piroplasms, seroconversion for *T. annulata* and *Hyalomma* tick infestation in different breeds of cattle

Breed	Sample size	Animal season at risk ^a	Detection of piroplasm IR ^b	Animal season at risk ^a	Sero-conversion IR	Tick infestation (%)
Holstein	76	89.00	0.449	105.00	0.180	25.0
Cross	67	78.85	0.557	111.28	0.116	20.9
Local	12	13.57	0.589	21.28	0.093	50.0

^aNo. of animal months at risk (April–October)/no. of months

^bIR, incidence rate

Variation in management between villages

In the two villages in which the cattle were not moved regularly to pasture but were usually fed indoors during the disease season, only 2 of 17 animals (11.7%) became parasitaemic and none was seropositive for *T. annulata*. No *Hyalomma* ticks were found on the animals or in the houses. In the other 10 villages, in which cattle were moved regularly to pasture during the disease season, 90 of the 138 cattle (65.2%) showed piroplasms and 34 (24.6%) were seropositive for *T. annulata*. *Hyalomma* ticks were found on 39 cattle (28.2%) and in two barns. Table III shows the tick infestations, Table VI shows the prevalence and incidence of piroplasms and the seroprevalence and incidence of seroconversion for *T. annulata* in cattle in the two groups of villages in which different management systems were practised.

TABLE VI

Prevalence and incidence of carriers of piroplasms, seroprevalence and incidence of seroconversion for *T. annulata* in the animals in the two types of village

	Two zero-grazed management systems	Ten standard management systems
Prevalence of carriers of piroplasms	0.010	0.101
Seroprevalence of <i>T. annulata</i>	0.000	0.106
Incidence of piroplasm carriers	0.340	0.535
Incidence of seroconversion for <i>T. annulata</i>	0.000	0.169
Percentage of infestation with ticks	0.000	28.20

DISCUSSION

The results confirmed that four species of *Hyalomma* ticks – *H. a. anatolicum*, *H. a. excavatum*, *H. detritum* and *H. m. marginatum* – occur in Central Anatolia (Kurtpinar, 1954; Mimioglu, 1954) and that all four species can transmit *T. annulata* from cattle to cattle in the field (Stepanova and Zablotskii, 1989).

On the basis of the climate of the Ankara area and from published data regarding climate and development periods for *H. a. anatolicum* (Snow, 1969; Pomerantzev, 1959; Walker *et al.*, 1985), *H. a. anatolicum* (Berdyev, 1974), *H. detritum* (Sergeant *et al.*, 1945; Pomerantzev, 1959; Karaer, 1981; Flach and Ouhelli, 1992) and *H. m. marginatum* (Nuttall, 1915, cited in Hoogstraal, 1956) it may be assumed that engorgement of the ticks on cattle and egg laying by female *Hyalomma* ticks take place in June and July. The eggs develop during July and August, and the larvae hatch and attach to cattle in August, before engorging and moulting into nymphs in September. The nymphs engorge on the cattle and drop off in October and then moult into unfed adults in April and May. In the barns, they moult into adults during November and overwinter in cracks and crevices in the walls of the barn. *Hyalomma detritum* acts as a two-host tick on cattle (Sergeant *et al.*, 1945; Hoogstraal, 1956; Pomerantzev, 1959), whereas *H. a. anatolicum*, *H. a. excavatum* and *H. m. marginatum* all act as three-host ticks (Hoogstraal, 1956; Pomerantzev, 1959).

Cases of theileriosis occur in late spring and summer, soon after the appearance of adult *H. a. anatolicum*, *H. detritum*, *H. a. excavatum* and *H. m. marginatum*. Larvae and nymphs of these tick species become infected with *T. annulata* when they feed on carrier animals in the autumn. The ticks overwinter as engorged nymphs and the susceptible cattle become infected with *T. annulata* when the infected, unfed adult ticks feed on the cattle in late spring and early summer. Experimental studies have shown that unfed nymphs of *H. detritum*, *H. a. anatolicum*, *H. a. excavatum* and *H. m. marginatum* became infected when they fed on *T. annulata*-infected calves (Sayin *et al.*, 1997) and unfed *T. annulata*-infected nymphs of *H. detritum* (Tasci, 1984) and *H. a.*

excavatum (Dumanli, 1987) could transmit the parasite to susceptible calves after moulting into adults. It was also reported that, experimentally, *T. annulata*-infected nymphs of *H. detritum* (Gill *et al.*, 1974), *H. a. anatolicum* and *H. m. marginatum* (Bhattacharyulu *et al.*, 1975) could transmit *T. annulata* to susceptible calves after moulting into adults. During this study, theilerial masses were found in the salivary glands of unfed adult *H. a. anatolicum*, *H. a. excavatum*, *H. detritum* and *H. m. marginatum* collected from cattle in early spring and summer in 37 (10+27) villages.

The life cycle of *T. annulata* proceeds within the carrier cattle during the summer; the immature ticks feeding on these cattle during the autumn, some as early as August, which may cause the cattle to react clinically. The parasites spend the winter as zygotes in engorged nymphs. The further development to kinetes and then sporoblasts occurs when the nymphs moult, then attach and feed as adult ticks in the late spring and early summer. The importance of carrier cattle in transmitting the infection to ticks was evident from this study. No clinical cases of *T. annulata* infection were seen during the feeding period of the immature ticks and, although rare clinical cases of *T. annulata* infection occur in late summer, the parasitaemia in newly infected animals falls within the seasonal range defined for carrier animals.

The probable reason for local breeds being more heavily infested with ticks than crossbreeds of Holsteins is that the cattle of the local breeds were not milked and spent much more time on the pasture, so that they did not benefit from inspection by the owners. Both the prevalence of tick infestation and the incidence of the appearance of piroplasms in the local breeds were greater than in the crossbreeds and Holsteins. The probable reason for this was the heavier tick infestation occurring on the cattle of the local breed. On the other hand, as the incidence of the appearance of piroplasms was higher than that of seroconversion for *T. annulata* in all breeds, it appears that either the IFAT is not sensitive enough to detect the *Theileria* antibodies or more than one *Theileria* species or strain of *T. annulata* was causing infection in the animals. This other species is probably *Theileria buffeli/orientalis*, as this is transmitted by the ticks of the genus *Haemophysalis*, which are widespread in Turkey.

The management of the animals in the two villages in which cattle were not moved to pasture but were fed indoors during the disease season appeared to have reduced the prevalence of tick infestation, the appearance of piroplasms, the seroprevalence of *T. annulata* infection, the incidence of the appearance of piroplasms and the incidence of seroconversion for *T. annulata* (Table VI). Both the seroprevalence and the incidence of seroconversion for *T. annulata* infection in the animals of both groups were significantly lower than the prevalence of the appearance of piroplasms.

Previously published articles have stated that *Hyalomma aegyptium*, *H. dromedarii*, *H. a. excavatum*, *H. a. anatolicum*, *H. detritum* and *H. m. marginatum* are widespread in Turkey (Kurtpinar, 1954; Mimioglu, 1954; Merdivenci, 1969; Khan, 1972). *Hyalomma detritum* transmits *T. annulata* between cattle in Israel (Pipano, 1976), Algeria (Sergeant *et al.*, 1945), Morocco (Flach and Ouhelli, 1992) and the USSR (Stepanova and Zablotskii, 1989) and both *H. a. anatolicum* and *H. a. excavatum* transmit the parasite in the USSR (Stepanova and Zablotskii, 1989). However, it was stated by Pipano (1977) that, while *H. a. excavatum* can transmit *T. annulata* experimentally, it does not do so under field conditions in Israel, as the immature forms of this tick feed on small

rodents and only the adults feed on cattle. In this present study, when almost replete adult ticks of both *H. a. excavatum* and *H. m. marginatum* were collected from cattle, and their salivary glands were dissected out and stained with methyl-green/pyronin, masses of *Theileria* parasite were revealed.

It was reported by Sergent and colleagues (1945) that fed nymphs and unfed adults of *H. detritum* were found in barns in Algeria where cattle became infected with tropical theileriosis. Studies in Turkey (Karaer, 1981) have shown that both adults and nymphs of this species of tick are found in cattle barns. However, in this present study, *H. detritum* ticks were not found in barns in the region around Ankara, although they were observed on cattle. In September, cattle housed in barns were found to be infested with large numbers of *H. a. excavatum* and *H. a. anatolicum* nymphs. When these barns were examined for the presence of unfed adult ticks between October and April, unfed adult *H. a. excavatum* and unfed adult *H. a. anatolicum* were found, but none were infected.

In spring, in parallel with the increasing levels of tick infestation, cattle infected with tropical theileriosis began to appear and their numbers increased throughout June to a peak in July. The study also revealed that the number of adult animals infected with *T. annulata* was much greater than the number of calves infected with the parasite. This is probably due to the fact that tick infestation is greater in the older animals. During the study period, 31% of adult cattle and 2.5% of calves became infested with *Hyalomma* ticks. Three ticks were found on one calf, but the number varied between 1 and 10 in older cattle, 63% being infested with more than 5 ticks.

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Études sur l'épidémiologie de la théilériose tropicale (Infection par *Theileria annulata*) chez le bétail de l'Anatolie centrale, en Turquie

Résumé – Une enquête épidémiologique portant sur les infections par *Theileria annulata* a été entreprise dans 12 villages sélectionnés situés aux environs d'Ankara de l'Anatolie centrale, en Turquie, durant la période allant d'avril 1990 à janvier 1993. Durant l'étude, 198 bovins de 30 races locales, 84 de race Holstein-Frisonne × races locales et 84 de race Holstein-Frisonne, ont été examinés pour déterminer la présence d'anticorps à *T. Annulata* et la présence de tiques vecteurs. Quatre espèces de tiques *Hyalomma* ont été identifiées : *Hyalomma anatolicum anatolicum*, *Hyalomma anatolicum excavatum*, *Hyalomma detritum* et *Hyalomma marginatum marginatum*. La coloration des glandes salivaires a indiqué que des tiques adultes infectées étaient présents dans les quatre espèces et étaient responsables de la transmission de la théilériose tropicale dans les champs. En règle générale, le taux d'infestation par *Hyalomma* a été bas, avec les infestations les plus sévères se produisant chez les animaux plus âgés. Les jeunes adultes et les veaux présentaient des taux d'infestation très bas. La plupart des tiques observés sur le bétail étaient des adultes, on a trouvé que très peu de nymphes. Le frottis sanguin et l'examen sérologique des 198 bovins effectué en mars avant le début de la première saison de la maladie a mis en évidence que la prévalence de la piroplasmose était de 11,1% (22 sur 198) et la séroprévalence de *T. Annulata* de 10,6% (21 sur 198). Quarante-trois animaux ont été exclus de l'étude car ils étaient séropositifs et/ou porteurs de piroplasmes. Quarante-vingt douze animaux séronégatifs présentaient une piroplasmose (92 sur 155) et 34 animaux séronégatifs sont devenus séropositifs à *T. Annulata* (34 sur 155) durant les trois saisons de la maladie. Un animal est devenu cliniquement malade avec une théilériose et a nécessité un traitement. Le pourcentage de bovins présentant une piroplasmose et une maladie dans l'ensemble de l'échantillon de l'étude a été de 50,7% et de 0,5% par saison de maladie, respectivement. Le taux de séroconversion de la nouvelle infection par *T. Annulata* dans l'ensemble de l'étude a été de 14,3% par saison animale. Le nombre de bovins présentant une piroplasmose a été supérieur au nombre de bovins séropositifs, ce qui pourrait indiquer la présence d'une autre espèce de *Theileria*. Les deux différents systèmes de traitement rencontrés dans l'étude ont été considérés comme ayant influencé les taux d'infestation par les tiques.

Epidemiología de la teileriosis tropical bovina (infección por *Theileria annulata*) en Anatolia central, Turquía

Resumen – Entre abril de 1990 y enero de 1993 se realizó un estudio epidemiológico de la infección por *Theileria annulata* en doce localidades situadas alrededor de Ankara (Anatolia Central, Turquía). Se analizaron 198 vacas, de las cuales 30 eran de razas locales, 84 eran cruces de Holstein-Friesiain y razas locales y 84 eran Holstein-Friesiain, en busca de anticuerpos frente a *T. annulata* y de las garrapatas que actúan como sus vectores. Se identificaron cuatro especies de *Hyalomma*: *Hyalomma anatolicum anatolicum*; *Hyalomma anatolicum excavatum*; *Hyalomma detritum* e *Hyalomma marginatum marginatum*. La tinción de las glándulas salivares reveló que las garrapatas adultas de las cuatro especies mencionadas estaban infectadas y que, por lo tanto, estaban implicadas en la transmisión de la teileriosis tropical. Por lo general, el grado de infestación por *Hyalomma* fue bajo, siendo los animales más viejos los que presentaban las infestaciones más importantes. Los adultos jóvenes y los terneros presentaron un grado de infestación muy bajo. La mayoría de garrapatas observadas en las vacas eran adultas, con presencia de muy pocas ninfas. En marzo, antes del inicio de la primera época de transmisión de la enfermedad, los análisis serológicos y el examen de los frotis sanguíneos de las 198 vacas revelaron una prevalencia de piroplasmosis del 11,1% (22 de 198) y una seroprevalencia de *T. annulata* del 10,6% (21 de 198). Se excluyeron del estudio 43 animales debido a que eran seropositivos y/o a que estaban infestados con piroplasmas. Noventa y dos animales seronegativos presentaron piroplasmas (92 de 155), mientras que 34 animales seronegativos pasaron a ser seropositivos frente a *T. annulata* (34 de 155) a lo largo de las tres épocas de transmisión de la enfermedad. Un animal presentó sintomatología clínica de teileriosis tropical y fue necesario tratarlo. La incidencia total de piroplasmas y de enfermedad en este estudio fue, respectivamente, del 50,7% y del 0,5% por época de transmisión. La tasa de seroconversión total del estudio frente a *T. annulata* fue del 14,3% por época de transmisión. El número de vacas con piroplasmas fue mucho mayor que el número de vacas seropositivas, lo que podría indicar que existe otra especie de *Theileria*. Los dos sistemas de manejo utilizados en el estudio influyeron en el grado de infestación por garrapatas.