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Original Article
Özgün Araştırma

Post-vaccination seroprevalence studies on the cattle vaccinated against Tropical theileriosis in Polatlı region[1]

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Abstract: This study was carried out to determine the effect of the Tropical theileriosis live schizont vaccine with the application in the field on cattle in Girmeç, Güreş, and Hıdırşeyh villages of Ankara province Polatlı district. For this purpose, the cattle in the villages were divided into 2 age groups under 1 year of age and over 1 year of age. From these, 280 cattle over 1 year of age and 35 cattle under 1 year of age were vaccinated. As the control groups, 37 cattle over 1 year of age and 70 cattle under 1 year of age were not vaccinated. After vaccination, blood was drawn from the vein jugularis of randomly selected cattle by regularly visiting the villages every month and their sera were taken for IFAT, and smears were prepared from blood samples collected from the ear tips of these animals. Seropositivity was 46.0% in the vaccinated under 1 year of age group and 29.1% in the control group. The differences between these two groups seropositivity were statistically insignificant (p=0.164). In the cattle over one year of age, seropositivity was 55.6 % in the vaccinated and 15.4% in the control group. The differences between these groups were found to be statistically significant (p=0.001). The rate of proplasm carrier cattle in the groups under 1 year of age is 13.5% in the vaccinated and 13.6% in the control group. The differences between these groups were not statistically significant (p=0.689). In the groups over 1 year of age, these rates are 24.7% in vaccinated cattle and 10.3% in the control group. The differences between these groups were also statistically insignificant (p=0.062). No serious complications were observed in any of the vaccinated groups. Clinical theileriosis was observed only in 3 (1 cow and 2 calves) of the vaccinated cattle. While 2 of the infected animals recovered without any treatment, 1 calf died. As a result, it was concluded that the region was stable for Tropical theileriosis thus, the cattle and newborn calves that would be introduced to this region should be vaccinated.

Keywords: Ankara Polatlı region, cattle, prevalence, Theileria annulata, vaccine.

Polatlı yöresinde Tropikal theileriosis'e karşı aşılanmış sığırlarda aşı sonrası seroprevalans çalışmaları

Özet: Bu çalışma Ankara ili Polatlı ilçesine bağlı Girmeç, Güreş ve Hıdırşeyh Köylerinde Tropikal theileriosis canlı şizont aşısının sahadaki uygulanış şekliyle sığırlar üzerindeki etkisini saptamak amacıyla yapılmıştır. Bunun için öncelikle köylerde bulunan sığırlar 1 yaş altı ve 1 yaş üstü olmak üzere 2 yaş grubuna ayrılmıştır. Bunlardan 1 yaş üstü 280 ve 1 yaş altı gruptan 35 sığır aşılanmıştır. Kontrol grubu olarak ise 1 yaş üstü 37 ve 1 yaş altı 70 sığır aşılanmamıştır. Aşılama sonrası her ay düzenli bir şekilde köylere gidilerek rastgele seçilen sığırların vena jugularis'inden kan alınmış ve IFAT için serumları çıkarılmış ve kulak ucundan alınan kan ile frotiler hazırlanmıştır. Bir yaş altı gruplarda seropozitivite aşılılarda %46,0 ve kontrol grubunda ise %29,1 olarak tespit edilmiştir. Bu iki grup arasındaki farklar istatistiki olarak önemsiz (p=0,164) bulunmuştur. Bir yaş üstü sığırlarda seropozitivite aşılılarda %55,6 ve kontrol grubunda %15,4 olarak bulunmuştur. Bu gruplar arasındaki farklılıklar istatistiki olarak önemli (p=0,001) bulunmuştur. Bir yaş altı gruplarda piroplasm taşıyıcı sığırların oranı aşılılarda %13,5 ve kontrol grubunda ise %13,6 olarak saptanmıştır. Gruplar arasında görülen farklılıklar istatistiksel öneme (p=0,689) sahip değildir. Bir yaş üstü sığırlarda bu oranlar aşılarda %24,7 ve kontrol grubunda ise %10,3'dır. Bu gruplar arasındaki farklılıklar da istatistiki yönden önemsizdir (p=0,062). Aşılama sonrası grupların hiç birisinde kayda değer komplikasyonlar görülmemiştir. Aşılanan sığırların 3 (1 inek ve 2 buzağı) tanesinde klinik theileriosis izlenmiştir. Hastalık görülen hayvanların 2 tanesi tedavi uygulanmaksızın iyileşirken 1 buzağı ölmüştür. Sonuç olarak, bölgenin Tropikal theileriosis için stabil olduğu tespit edilmiştir bu nedenle bu bölgeye steril bölgelerden getirilecek sığırlar ile yeni doğmuş buzağılar aşılanmalıdır.

Anahtar kelimeler: Ankara Polatlı yöresi, aşı, prevalans, sığır, Theileria annulata.

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Introduction

Theileriosis is a disease caused by obligate intracellular protozoa of Theileria spp. and it adversely affects cattle, sheep, goat, buffalo and zebu breeding, and livestock development, especially in Africa; in Southern Europe; in Australia, in Asia like Turkey, the Middle East, India and China (Levine, 1973; Dinçer, 1985; Kirvar, 1991; Morrison, 1998; Wilkie et al., 1998; Ahmed and Mehlhorn, 1999; Campbell and Spooner, 1999). The disease caused by *Theileria annulata* is called Tropical theileriosis and also called Tropical gonderiosis, Tropical piroplasmosis, Egyptian Fever, or Mediterranean Coast Fever (Mimioğlu et al., 1971; Dinçer, 1985; Mimioğlu, 1985; Flach and Ouhelli, 1992; Beniwal et al., 1997; Brown, 1997; Campbell et al., 1997).

The disease was first identified in Turkey in 1930-31 by the studies of İbrahim Ekrem Erbin (Unat et al. 1965). *T. annulata* is present all over Turkey and the disease especially affects culture and crossbred cattle breeds, and the mortality rate can reach 50% in domestic breeds and 100% in culture and crossbreeds (Sayın, 1991; Sayın et al. 1991). The mortality rate was detected to be 40-90% and varied from country to country (Morrison, 1998; Campbell & Spooner, 1999 and İnci et al., 2008).

It is reported that the economic loss in the world cattle breeding caused by diseases such as theileriosis, babesiosis and anaplasmosis transmitted by ticks is approximately 7 billion US Dollars (Brown, 1997). In developing countries, it was determined that 250 million cattle were at risk from *T. annulata* in 1982 (Brown, 1997; Campbell ve Spooner, 1999). In a study by İnci et al. (2001) in Turkey, it was reported that the disease caused an economic loss of approximately 70,000 US Dollars in two theileriosis seasons only in Develi, İncesu, Yahyalı and Yeşilhisar districts of Kayseri province. In a study conducted in Pakistan, Rashid et al. (2018) determined that the economic losses caused by Tropical theileriosis constitute 13.8% of the losses in livestock.

The disease has a seasonal course depending on the tick biology and clinical infections are mostly seen in June, July and August in Turkey. In addition, the parasite can be encountered latently in all seasons (Nalbantoğlu, 1998a; Ceylan et al. 2021). Hyalomma ticks play an important role in the transmission of tropical theileriosis. It was determined that approximately 15 species of ticks naturally and experimentally transmitted this parasite (Karaer, 1985; Nalbantoğlu, 1998b; Angın and Dumanlı, 1999).

Hyalomma ticks are very common in Turkey. In the studies, it was determined that *H. detritum, H. aegyptium, H. excavatum H. marginatum* are present in Turkey and the infestation rate with Ixodid ticks in cattle was found to be 34%. It has been observed that Hyalomma spp. ticks are very common, especially in the summer months (Merdivenci, 1969; Karaer, 1985; Sayın, 1991).

Aktaş et al. (2004) detected four different Hyalomma species (*H. a. anatolicum, H. a. excavatum, H. detritum* and *H.m. marginatum*) in cattle in eastern Turkey, and they detected natural Theileria infection in three of them. Among these tick species, *H. a. anatolicum* had the highest infection prevalence and density in Turkey. Selim et al. (2022) determined by risk factor analysis that the level of tick infestation plays a critical role in the prevalence of infection in older cattle, especially in the autumn and summer seasons.

A total of 51 tick species, 43 from the Ixodidae family and 8 from the Argasidae family, were identified in Turkey. The most important species are included in nine species such as Dermacentor, Haemaphysalis, Hyalomma, Ixodes, Rhipicephalus (Boophilus), Amblyomma, Argas, Otobius and Ornithodorus (Ceylan et al., 2021).

Many factors affect the epidemiology of the disease, such as host species, environmental conditions, temperature, vector ticks, carrier animals, age of animals and breeds of animal (Mimioğlu et al., 1971; Innes et al., 1992).

In the diagnosis of theileriosis, epidemiological information, clinical symptoms, laboratory examinations, serological tests (IFAT, ELISA) and PCR are commonly used (Göksu, 1985; OIE Manual, 1996; Bilgiç et al. 2013; OIE Website, 2022). Serological tests are used to diagnose the disease, especially in the diagnosis of latent infections with low parasitemia, and thus, the use of these tests is increasing in epidemiological studies (Kachani et al., 1996; Eren et al., 1998). Although acute theileriosis can be easily diagnosed by the appearance of macroschizonts in lymph node biopsy preparations stained with Giemsa, it is difficult to diagnose chronic cases due to the small number of proplasms seen in blood smears. Also, it is often not easy to distinguish different Theileria species. Therefore, serological tests are preferably used in the diagnosis of theileriosis (Ahmed et al., 1997).

Animals that recovered from *T. annulata* infection following natural transmission by ticks became immune to reinfection by the parasite (Özkoç, 1985;

Ahmed and Mehlhorn, 1999; Ahmed et al. 1999b; Caccio et al. 2000). It is thought that this immunity provides varying degrees of protection against heterologous strains of the parasite (Preston and Jackson, 1991; Ahmed et al. 1999a).

Immunologically, there is a critical balance between the parasite and the host. The parasite controls the activity of the immune system and the immune system controls the reproduction of the parasite. If the balance changes in favor of any side, the results develop in favor of that side (Innes, 1991; Innes et al. 1992).

The control of the disease is based on the improvement of tick control, chemotherapy, immunization and care-feeding methods (Singh, 1990). In addition to these, epidemiological studies should be conducted to determine the incidence of vector ticks and the biological characteristics of Hyalomma species in Turkey. Enzootic regions should be identified and the role of buffaloes in the transmission of the disease should also be investigated (Sayın, 1985).

A live schizont vaccine developed against this disease is used in many countries to control the disease, (Flach and Ouhelli, 1992; Innes et al., 1992; Beniwal et al., 2000; Sayın et al., 2004). Vaccination is seen as the most effective control method. Animals that have recovered from the disease are immune to this disease for a long time and are not affected (Innes et al., 1992; Pipano, 1994; Sayın et al., 2004).

The epidemiological characteristics of the region should be revealed to plan the vaccine to be used where and when. Studies have shown that endemic, stable and unstable regions are found; accordingly, although it is a factor in both the vector tick and the host in a region, the absence of clinical cases indicates that the region is endemic stable. In such regions, it is sufficient to vaccinate only externally introduced animals and newborn calves. In unstable areas, that is, if clinical cases are frequently seen in animals over 3 years of age; in this case, all animals should be vaccinated.

This study was carried out to investigate the effects of the live schizont tissue culture vaccine produced against Tropical theileriosis in cattle in Turkey with its application in the field, immunity created by this vaccine and epidemiology of the disease after vaccination.

Material and Method

Field Studies

Selection of Villages

This study was carried out in Girmeç, Güreş and Hıdırşeyh villages of Ankara Polatlı district where crossbred and culture cattle breeds are common. The villages are 60 km away from Ankara and the distance between the villages is about 5-15 km. Among these villages, Güreş had 75, Girmeç 50 and Hıdırseyh 25 households. Their altitudes are almost the same as Ankara, 750-800 meters, and the climate of the region reflects the typical continental climate of Central Anatolia.

Animal husbandry was mostly in the form of traditional small family businesses. There were also very few medium-sized businesses and modern reinforced concrete shelters. Most of the barns were made of adobe, lacking adequate ventilation, suitable for ticks, with bad floors and unplastered walls. Along with cattle breeding, a significant amount of sheep and poultry (chicken and turkey) breeding was also carried out in the villages.

The majority of the cattle population consisted of the Holstein breed and their crosses. Breeding was generally in the style of dairy cattle and, beef cattle breeding was also carried out. The breeding of cattle was mostly carried out by artificial insemination in Girmeç and Hıdırşeyh, and by village bull (natural mating) in Güreş.

In Girmeç and Güreş villages, animals were fed in the form of a village herd by grazing in the pasture from early morning to late evening from March to November. On the other hand, animals were not released to the pasture in Hıdırşeyh, but were grazed around the village by their owners.

There were 422 cattle including 244 in Güreş, 99 in Girmeç and 79 in Hıdırşeyh villages. These cattle were divided into two groups according to their age, as Group 1 (under 1 year of age) and Group 2 (over 1 year of age). The cattle vaccinated and left as control according to age groups in villages are shown in Table 1.

In Table 1, there were 48 cattle in Group 1 and 196 in Group 2 in Güreş. There were 41 cattle in Group 1 and 58 in Group 2 in Girmeç, and 16 cattle in Group 1 and 63 in Group 2 in Hıdırseyh. In April, which was the same time as the practice in the field, 14 cattle from Group 1 and 174 cattle from Group 2 in Güreş were vaccinated. In Girmeç, 15 cattle from Group 1 and 47 cattle from Group 2 were vaccinat-

ed. And in Hıdırseyh village, 6 cattle from Group 1 and 59 cattle from Group 2 were vaccinated. A total of 35 cattle from Group 1 and 280 cattle from Group 2 were vaccinated in these 3 villages.

Table 1. The number of vaccinated and unvaccinated cattle according to age groups in Güreş, Girmeç and Hıdırşeyh villages.

Villages	Vaccinated		Control		Group 1 Total	Group 2 Total	Overall Total
	Group 1	Group 2	Group 1	Group 2			
Güreş	14	174	34	22	48	196	244
Girmeç	15	47	26	11	41	58	99
Hıdırşeyh	6	59	10	4	16	63	79
Total	**35**	**280**	**70**	**37**	**105**	**317**	**422**

As the control group, there were 34 cattle from Group 1 and 22 from Group 2 in Güreş village, 26 cattle from Group 1 and 11 from Group 2 in Girmeç, 10 cattle from Group 1 and 4 from Group 2 in Hıdırseyh. A total of 70 cattle from Group 1 and 37 cattle from Group 2 were not vaccinated.

To determine the initial prevalence of the disease in cattle grouped in this way, blood was taken from 27 cattle from Group 1 and 32 from Group 2, out of total 59 cattle before vaccination. It was determined if they had antibodies against the disease by IFAT, and proplasm and schizont forms were searched by microscopic examination in the smears prepared from the same animals.

After the vaccination, the villages were visited every month. From the groups formed, 44-83 cattle were randomly selected and blood was drawn for antibody detection in the serological examination, and thin smears were made from the blood sample taken from the ear tips to detect carrier animals.

IFAT was performed to detect antibodies against *T. annulata* in blood serum. The proplasm forms of the agent were determined by microscopic examination of the smears (200 microscope fields were examined in each smear).

In vaccinated cattle, 1 year examination results were evaluated in the first 6 months and the second 6 months, taking into account the effective period of vaccine as 6 months stated in the vaccine instructions.

In every visit to the villages, it was asked if there were any sick cattle and if there were, blood was taken for IFAT and smears were made to detect proplasmic forms, and lymph node puncture was performed to detect schizonts.

Vaccine Administration

T. annulata live attenuated cell culture vaccine produced by the Pendik Veterinary Control Institute of the Ministry of Agriculture and Forestry was used to vaccinate cattle in the villages. The vaccine was prepared by making 250 passages in monolayer cell culture from Ankara stock of *T. annulata* and was a live vaccine. For this reason, it was brought from the producer institute and was taken to the villages in a Liquid Nitrogen tank (-196 °C). Vaccination was done according to vaccine producer instructions in early April when ticks were not yet active.

Laboratory Studies

The blood samples were brought to the laboratory within a few hours, centrifuged at 1500-2000 rpm for 10 minutes and their sera were extracted. These sera were taken into 1.5 ml tubes, labeled and stored in a deep freezer at -20°C until the IFA test.

Indirect Fluorescent Antibody Test

Since proplasmic forms of *T. annulata* cannot be produced in tissue culture, piroplasm antigens must be obtained from infected animals. The piroplasm antigen required for the test was produced at Ankara University, Faculty of Veterinary Medicine, Department of Protozoology-Entomology (Çakmak, 1987; OIE Manual, 1996).

The schizont antigen was also obtained from *T. annulata* cell culture in the same Department Tissue Culture Laboratory (Çakmak, 1987).

Rabbit-Bovine IgG (Sigma, Anti-bovine IgG, Cat. No: F-7509) labeled with FITC (Fluorescent Isothiocyanate) was used to be the conjugate (Çakmak, 1987).

As the positive control serum, the serum obtained from the blood collected on the 42nd day from a calf infected with *T. annulata* Gülseren strain in the same Department was used. The negative serum required for the test was obtained from the same experimental calf before it was infected with *T. annulata*.

PBS was used as buffer control (pH=7.2). Again, the conjugate diluted 1:32 with a mixture of 3 parts PBS+1 part Evans Blue was used as the conjugate control. The addition of Evans Blue makes it easier to read (Çakmak, 1987).

In this test, the baseline titer was taken as 1:20 for *T. annulata* proplasm antigen and 1:40 for schizont antigen.

The sera to be processed were removed from -20 °C one day in advance and taken to +4 °C. The previously prepared antigen preparations were taken from -70 °C and placed in a closed container with a desiccant (CaCl2) and left for 2 hours. The sera were diluted with PBS between 1:10-1:1280 in microtitration plates.

The preparations were examined with a fluorescent microscope in the dark room with a 40x neofluar objective.

Examination of Blood Smears

Blood smears prepared from the blood taken from the ear tips of the animals were fixed in commercial methyl alcohol for 5 minutes and then stained with 5% Giemsa dye (diluted with PBS pH 7.2) for 45 minutes. The stained preparations were examined by dropping oil immersion by 100x objective. 200 microscope fields were examined in each preparation and proplasmic forms were counted.

Statistical Evaluations of Results

Statistical calculations were made using the SYSTAT (Systat Version 5.0, Systat, Inc.) program. The significance of the difference between the vaccinated and control groups was compared with the "paired t-test".

Results

Pre-vaccination Serological and Microscopic Findings

Serological and microscopic examination results according to age groups before vaccination are shown in Table 2. Accordingly, *T. annulata* antibodies were detected by IFAT in 1 (0.4%) of 27 cattle in Group 1 and 6 of 32 cattle (18.8%) in Group 2.

One of 27 cattle (0.4%) in Group 1, and 12 (37.5%) of 32 cattle in Group 2, were found to be carriers of proplasm by microscopic examination.

Table 2. Serological and microscopic examination results by age groups before vaccination.

Age Groups	Number of cattle	Seropositivity	Piroplasm
Group 1	27	1 (0.4%)	1 (0.4%)
Group 2	32	6 (18.8%)	12 (37.5%)
Total	**59**	**7 (11.9%)**	**13 (22.0%)**

Post-Vaccination Serological and Microscopic Findings

Serological Findings

Seropositivity in Vaccinated Cattle

After the vaccination in the villages, monthly serological examination results are given in Table 3. As seen in the table, monthly seropositivity was found to be between 14.3-88.9% in Group 1 and 22.5-77.8% in Group 2 during the 12 months of study. These rates were determined within the first 6 months when the vaccine was effective. Within the second 6 months period, seropositivity was found to be 16.7-71.4% in Group 1 and 47.1-77.1% in Group 2.

Table 3. Monthly post-vaccination serological examination results and percentage rates in vaccinated cattle, according to age groups.

Months	Group 1		Group 2	
	Seropositivity		Seropositivity	
	*	%	*	%
May	8/9	88,9	22/46	47,8
June	1/7	14,3	11/31	35,5
July	6/13	46,2	28/36	77,8
August	13/21	61,9	28/43	65,1
September	1/7	14,3	13/49	22,5
October	3/17	17,6	25/45	55,6
November	2/5	40,0	27/35	77,1
December	5/7	71,4	18/30	60,0
January	2/5	40,0	23/37	62,2
February	7/10	70,0	19/30	63,3
March	1/6	16,7	24/41	58,5
April	1/4	25,0	16/34	47,1
Total	**51/111**	**46,0**	**254/457**	**55,6**

*: Number of cattle with the antibodies against *T. annulata* (seropositive) / number of cattle examined.

Seropositivity in Control Group Cattle

Table 4 shows seropositivity in control group cattle. As seen from this table, seropositivity was found to be 0-47.4% in Group 1 and 0-20% in Group 2 between April and November, the disease season in

Central Anatolia. In the months between November and April after the disease season, seropositivity was seen to be 14.3-75% in Group 1 and 0-50% in Group 2.

Table 4. Monthly serological examination results and percentage rates according to age groups in unvaccinated (control) cattle.

Months	Group 1		Group 2	
	Seropositivity		Seropositivity	
	*	%	*	%
May	0/3	0.0	0/1	0.0
June	1/6	16.7	1/8	12.5
July	6/21	28.5	0/3	0.0
August	9/19	47.4	-	-
September	0/11	0.0	2/10	20.0
October	2/10	20.0	0/1	0.0
November	2/8	25.0	-	-
December	2/3	66.7	2/4	50.0
January	2/6	33.3	1/5	20.0
February	3/4	75.0	0/5	0.0
March	2/5	40.0	0/2	0.0
April	1/7	14.3	-	-
Total	**30/103**	**29.1**	**6/39**	**15.4**

* : Number of cattle with the antibodies against *T. annulata* (seropositive) / number of cattle examined.

It was found that the difference in seropositivity between the vaccinated and control groups was not statistically significant (p=0.164) while the difference in seropositivity was statistically significant (p=0.001) between Group 2s.

Serum Antibody Levels

The monthly distribution of the lowest and highest antibody titers determined by IFAT against proplasm and schizont antigens belonging to the vaccinated and control groups are shown in Tables 5 and 6.

In Table 5, antibody titration against proplasm antigen varied between 1:20-1:1280 in Group 1 and 1:20-1:640 in Group 2 in the first 6 months when the vaccine was effective in the vaccinated groups. In the next 6 months, it was seen that the antibody titers were between 1:20 and 1:320 in Group 1 and Group 2.

In the control groups, the 1-year antibody titers against proplasm antigen were found to be 0-1:320 in Group 1 and 0-1:160 in Group 2 (Table 5).

In Table 6, antibody titers against schizont antigen were between 0-1:640 in Group 1and 1:40-1:640 in Group 2 in the first 6 months when the vaccine was effective in the vaccinated groups. In the following 6 months, the antibody titers were found to be 0-1:160 in Group 1 and 0-1:640 in Group 2.

In the control groups, antibody titers against schizont antigen were detected to be 0-1:320 in Group 1 and 0-1:40 in Group 2 (Table 6).

Table 5. The highest and lowest antibody titers detected by proplasm antigen in vaccinated and control group cattle.

Age	Titer	May	June	July	August	September	October	November	December	January	February	March	April
Group 1 Vaccinated	LAT	1:40	1:160	1:20	1:20	1:640	1:40	1:20	1:40	1:40	1:40	1:160	1:40
	HAT	1:80	1:160	1:640	1:1280	1:640	1:640	1:320	1:160	1:40	1:40	1:160	1:160
Group 1 Control	LAT	0	1:80	1:20	1:20	0	1:80	1:40	1:40	1:40	1:40	1:40	1:40
	HAT	0	1:80	1:40	1:320	0	1:80	1:160	1:40	1:80	1:160	1:80	1:40
Group 2 Vaccinated	LAT	1:40	1:40	1:20	1:20	1:20	1:20	1:20	1:40	1:40	1:40	1:40	1:40
	HAT	1:320	1:640	1:640	1:640	1:320	1:320	1:320	1:40	1:160	1:80	1:160	1:160
Group 2 Control	LAT	0	1:40	0	-	1:40	0	-	1:40	1:160	0	0	-
	HAT	0	1:40	0	-	1:40	0	-	1:40	1:160	0	0	-

LAT: The Lowest Antibody Titer HAT: The Highest Antibody Titer

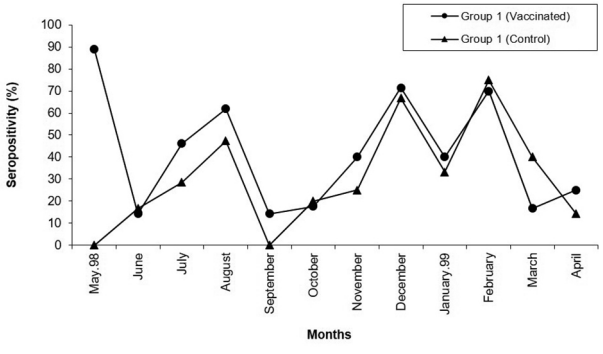
Table 6. The highest and lowest antibody titers detected with schizont antigen in vaccinated and control cattle.

Age	Titer	May	June	July	August	September	October	November	December	January	February	March	April
Group 1 Vaccinated	LAT	0	0	1:40	1:160	0	1:80	1:160	0	0	0	0	1:80
	HAT	0	0	1:80	1:640	0	1:80	1:160	0	0	0	0	1:160
Group 1 Control	LAT	0	1:40	1:80	1:80	0	1:40	1:40	0	0	0	0	1:160
	HAT	0	1:40	1:160	1:320	0	1:40	1:40	0	0	0	0	1:160
Group 2 Vaccinated	LAT	1:40	1:40	1:40	1:40	1:80	1:40	1:40	1:80	1:160	0	1:40	1:40
	HAT	1:160	1:160	1:640	1:640	1:160	1:160	1:640	1:160	1:160	0	1:80	1:320
Group 2 Control	LAT	0	1:40	0	-	1:40	0	-	0	0	0	0	-
	HAT	0	1:40	0	-	1:40	0	-	0	0	0	0	-

LAT: The Lowest Antibody Titer HAT: The Highest Antibody Titer

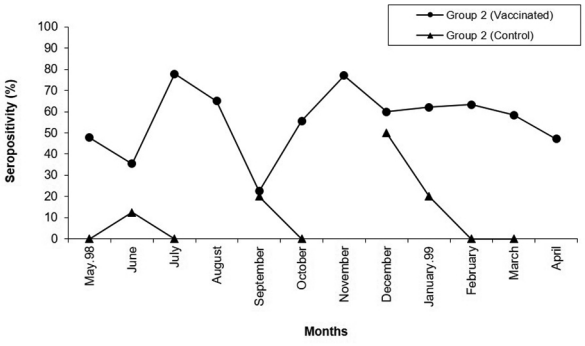
Seropositivity

The seropositivity in the vaccinated and control groups was shown in Graph 1 and Graph 2. Graph 1 showed the course of seropositivity of vaccinated and unvaccinated cattle in Group 1. In these groups, the initial May seropositivity was 88.9% in vaccinated cattle. In unvaccinated cattle, except for 0%, the seropositivity was similar in both the vaccinated and control groups during the other 11 months. And, during the 1-year study period, August, December, and February were the months when seropositivity increased in both vaccinated and unvaccinated cattle (Graph 1).



Graph 1. Seropositivity of vaccinated and unvaccinated cattle in Group 1.

Graph 2 shows the course of seropositivity in Group 2 vaccinated and control cattle. As could be seen from this, the seropositivity of vaccinated cattle was higher in July (77.8%), August (65.1%) and November (77.1%) compared to other months, and the seropositivity was between 22.5-77.8% for 1 year. It was observed that the percentage of seropositivity was higher in the control group in June (12.5%), September (20.0%), December (50.0%) and January (20.0%) compared to other months, and no seropositivity was detected in February and March.



Graph 2. Seropositivity in Group 2 vaccinated and unvaccinated cattle.

An increase in seropositivity and antibody titers was noted in vaccinated cattle compared to control groups. This showed that the vaccine activated the immune system. The increase in seropositivity was not significant in the group below 1 year of age, and the difference with the control group was statistically insignificant (p=0.164). In contrast, there was a statistically significant (p=0.001) increase in seropositivity in vaccinated cattle in the 1-year-old group compared to the control group. The fluctuating increases in seropositivity and antibody titers did not decrease to zero in the other groups, except for the 1-year-old control group.

Microscopic Findings

Microscopic Findings in Vaccinated Cattle

The monthly proplasm carrier results after vaccination according to age groups in study villages were presented in Table 7. Proplasm forms were detected in 15 (13.5%) of 111 cattle in Group 1 and 113 (24.7%) of 457 cattle in Group 2 which were examined for one year (Table 7).

In Table 7, it was seen that the rate of carrying proplasm for 1 year varied between 0-71.4% in Group 1 and 0-60.0% in Group 2. It was seen that the rate of those carrying proplasm in the vaccinated Group 1 cattle in the first 6 months following the vaccination was between 0-19.1% and this rate was between 0-71.4% in the second 6-month period. In the vaccinated Group 2, the percentage of proplasm found in the first 6-month period after vaccination was between 2.8-58.1% and between 0-60.0% in the second 6-month period.

Table 7. Post-vaccination monthly proplasm carrier numbers and percentages in cattle vaccinated according to age groups.

Months	Group 1		Group 2	
	Carrier		Carrier	
	*	%	*	%
May	1/9	11,1	19/46	41,3
June	1/7	14,3	18/31	58,1
July	0/13	0,0	1/36	2,8
August	4/21	19,1	13/43	30,2
September	0/7	0,0	2/49	4,1
October	1/17	5,9	4/45	8,9
November	1/5	20,0	21/35	60,0
December	5/7	71,4	18/30	60,0
January	0/5	0,0	8/37	21,6
February	0/10	0,0	0/30	0,0
March	0/6	0,0	0/41	0,0
April	2/4	50,0	9/34	26,5
Total	**15/111**	**13,5**	**113/457**	**24,7**

* : Number of cattle with *T. annulata* pyroplasms / number of cattle examined.

Microscopic Findings in Control Group Cattle

Table 8 showed the numbers and percentages of cattle carrying proplasm in the control group. Accordingly, proplasm forms were detected in 14 (13.6%) of 103 cattle from Group 1 and 4 (10.3%) of 39 cattle from Group 2 for 1 year period.

As seen in the table, the rates of cattle carrying proplasm in the control Group 1 were 0-66.7% in the first 6 months after vaccination and 0-66.7% in the second 6 months. In the control Group 2, the percentage of cattle carrying proplasm was found to be between 0-37.5% in the first 6 months and 0-20.0% in the second 6 months.

It was determined that the differences between the vaccinated and control groups of Group 1s (p=0.689) and Group 2s (p=0.062) were not statistically significant.

Table 8. Monthly numbers and percentages of proplasm carriers by age groups in unvaccinated (control) cattle.

Months	Group 1		Group 2	
	Carrier		Carrier	
	*	%	*	%
May	2/3	66,7	0/1	0,0
June	1/6	16,7	3/8	37,5
July	1/21	4,8	0/3	0,0
August	5/19	26,3	-	-
September	0/11	0,0	0/10	0,0
October	0/10	0,0	0/1	0,0
November	2/8	25,0	-	-
December	2/3	66,7	0/4	0,0
January	0/6	0,0	1/5	20,0
February	0/4	0,0	0/5	0,0
March	0/5	0,0	0/2	0,0
April	1/7	14,3	-	-
Total	**14/103**	**13,6**	**4/39**	**10,3**

* : Number of cattle with *T. annulata* proplasms / number of cattle examined.

Parasitemia

The lowest and highest proplasm numbers detected in vaccinated and unvaccinated (Group 1 and Group 2) cattle in the study centers during 1 year period were shown in Table 9. According to this table, a maximum of 60 piroplasms were observed in one cattle in the first 6 months in vaccinated Group 1, and parasitemia in this group was observed to be between 0-0.11% in this period. In the second 6-month period, a maximum of 15 piroplasms were counted in 1 cattle and parasitemia was found to be between 0-0.03%.

In Group 1 cattle that were left as control, a maximum of 34 piroplasms were counted in 1 cattle and parasitemia was found to be between 0.0 and 0.06%.

In Group 2 vaccinated cattle, the highest number of piroplasms seen in 1 cattle in the first 6 months was 78 and parasitemia was between 0.002-0.15% in this group. In the second 6 months, the

highest piroplasm number seen in one cattle was 42, and parasitemia was observed to be between 0-0.08%.

In unvaccinated cattle in Group 2, maximum 20 proplasms were counted in 1 cattle and parasitemia was found to be 0-0.04%.

Table 9. The lowest and highest proplasm numbers in an animal in vaccinated and unvaccinated cattle.

Age	Titer	May	June	July	August	September	October	November	December	January	February	March	April
Group-1 Vaccinated	LPN	60	45	0	3	0	2	10	3	0	0	0	5
	HPN	60	45	0	26	0	2	10	6	0	0	0	15
Group-1 Control	LPN	2	34	3	4	0	0	10	1	0	0	0	24
	HPN	25	34	3	32	0	0	12	19	0	0	0	24
Grup-2 Vaccinated	LPN	2	4	76	1	2	2	2	1	1	0	0	3
	HPN	78	40	76	18	2	11	30	13	11	0	0	42
Group-2 Control	LPN	0	4	0	-	0	0	-	0	3	0	0	-
	HPN	0	20	0	-	0	0	-	0	3	0	0	-

LPN: The lowest proplasm number HPN: The highest proplasm number

Dıscussıon

Tropical theileriosis is a disease seen in all regions of Turkey and threatens cattle breeding to a great extent. This disease especially affects culture and crossbred cattle. The mortality rate can reach 50% in native breeds and 100% in culture and crossbreeds (Sayın, 1991; Sayın ve ark., 1991).

Diagnosis of the disease is made by clinical signs, laboratory studies, serological (IFAT, ELISA) and molecular tests (PCR). In the microscopic examination, the smears prepared from blood and lymph nodes are examined and the diagnosis is made by seeing proplasmic forms and especially schizonts (Göksu, 1985; OIE Manual, 1996; Ilchmann, 1989).

In this study, the prevalence of the disease was determined as 0.4% in the group under 1 year of age cattle and 37.5% in the group over 1 year of age by microscopic examination before vaccination. The percentage of proplasm carriers detected in cattle older than one year was very close to the rates found in Central Anatolia (37%) (Sayin, 1991) and the Black Sea region (34%) (Dinçer et al., 1991). The results also were between the values found by other researchers (0-64%).

In this study, the vaccine application was made similar to that of the field application, which was not administered only to seronegative cattle but to all. The proplasm carrier rate was determined as 11.1% in vaccinated cattle and 66.7% in unvaccinated cattle under 1 year of age. The rate of those carrying piroplasm after vaccination was 0-19.1% in cattle under 1 year of age in the first 6 months, and 0-71.4% in the second 6 months. In the control group under 1 year of age, the rate of those carrying piroplasm for 1 year period was found to be between 0-66.7%. Considering the microscopic prevalence by months, it followed a fluctuating course throughout the study.

Microscopy results showed a similar fluctuation in the vaccinated under 1 year of age group. In other words, in the months when one was high, the other was also high. In the vaccinated group, 13.5% of the cattle were found to be carriers of proplasm. In the control group, this rate was 13.6%. The results are almost the same and the difference between them is statistically insignificant (p=0.689).

It was determined that the percentage of proplasm carrier cattle, which was 37.5% before vaccination in the vaccinated over 1 year of age group, was 41.3% in the first month after vaccination. These rates were found to be between 2.8-58.1% in the first 6 months after vaccination and between 0-60% in the second 6 months.

In the control group cattle over 1 year of age, proplasm carrier rates were found to be between 0-37.5% in a year. After one-year study, the mean microscopic prevalence was found to be 24.7% in vaccinated cattle older than 1 year of age, while it was determined as 10.3% in the control group. According to this result, the rate of those carrying piroplasm in the vaccinated group is more than 2

times that of the control group cattle. However, the differences between the vaccinated group and the control group were statistically insignificant (p=0.062).

Tropical theileriosis can also be diagnosed with many serological tests developed in recent years. In Turkey, theileriosis was diagnosed for the first time by IFAT by Çakmak (1987). Subsequently, numerous studies have been conducted using IFAT.

In serological studies using IFAT; Sayin et al. (1991) detected *T. annulata* antibodies in 31% of cattle in the Ankara region; Dinçer et al. (1991) in 63% of cattle in Samsun; in another study by Sayın et al (1994) in 23%, 3% and 53% of cattle in Adana, Bursa and Elazığ, respectively. Zeybek et al (1994) detected the antibodies in 3% of cattle under 1 year of age and 12.9% of cattle over 1 year of age in Çankırı; Nalbantoğlu (1996) in 16% of the cattle from Çukurova Agricultural Enterprise in Adana province; Vatansever (1997) in 0% of the cattle before vaccination in Çukurova region; Eren et al. (1998) in 31% of cattle in Aydın region; and Açıcı (1998) in 1.9% of the cattle in Samsun region.

In this study, before vaccination, the seroprevalence was 18.8% in cattle older than 1 year of age and 0.4% in cattle under 1 year of age by IFAT. The result obtained in cattle over one year of age, was similar to the results of Sayın et al. (1994) in Central Anatolia, Nalbantoğlu (1996) in the Adana region, and Aktaş et al. (1999) in the Malatya region (23.3%; 20 and 17.1%). In addition, seropositivity was found between 0% (Vatansever, 1997; Karatepe, 2000) and 50.23% (İnci et al., 1999) in studies. Seroprevalence found in this study was between these values. It is thought that the difference between the results obtained in the studies is due to the factors affecting the prevalence of intermediate host ticks, the possibility of coming into contact with animals, regional humidity, temperature and altitude differences and breeding style.

To prevent losses caused by tropical theileriosis and to protect animals from this disease; immunogenic, protective and non-pathogenic *T. annulata* live schizont vaccine has been developed in many countries. In Turkey, live attenuated *T. annulata* cell culture vaccine was used to be produced by the Pendik Veterinary Control Institute but production was terminated over time. Today, the theileria vaccine is still produced in Turkey by a private companies. But to fully determine the immunity period of the vaccine is difficult because 1) vaccinated animals must be kept in a tick-free environment and 2) contact with ticks in endemic areas strengthens the resulting immunity (Singh, 1989; Beniwal et al., 1997; Beniwal et al., 2000).

In the incidence studies conducted by some researchers (Nalbantoğlu, 1996; Vatansever, 1997; Açıcı, 1998; Yaman, 1998; Öz, 1999; Karatepe, 2000) in different regions of Turkey, after vaccination of seronegative cattle, the antibody titers formed against iroplasm and schizont antigens were determined in cattle.

Of these, Nalbantoğlu (1996) vaccinated 58 seronegative cattle and found that 45 (77.6%) of them formed antibodies against piroplasm and schizont antigen. Seropositivity continued for 12 months, decreasing from the 4th month in the 0-1 age group following vaccination. However, in the 1-2 year of age group, the antibody titer started to decrease in the 3rd month and was zeroed in the 7th month. Similarly, in the age group over two years of age, it started to decrease in the 3rd month and was zeroed in the 4th month. Antibody titers against piroplasm antigen were found to be 1:20 and 1:5120, while titers against schizont antigen were observed as 1:40 and 1:2560. The highest piroplasm and schizont antibody titers were detected in the 0-1 year of age group in April and May. In the study, it is emphasized that this region is unstable and it is accepted that vaccination once a year will be beneficial for the control of the disease.

Vatansever (1997) in a study conducted in the Çukurova region, concluded that this region is unstable in terms of Tropical theileriosis, therefore it is necessary to fight against vector ticks and to continue vaccination studies.

Açıcı (1998) determined that antibodies were formed in 90.9% of 44 seronegative cattle after vaccination. The antibody titer in vaccinated cattle increased up to 1:2560. This study showed that the disease did not show an endemic course in the region and that the region was unstable. For this reason, it was concluded that vaccination is necessary for the prevention of the disease.

Yaman (1998) observed that in cattle vaccinated against tropical theileriosis, 79.62% of them were seropositive in the first probe following the vaccination. The seropositivity continued for 3 months, started to decrease from the 4th month and ended in the 7th month. Antibody titers in seropositive cattle were between 1:20 and 1:5120 against piroplasm antigens, and between 1:40 and 1:2560 against schizont antigens. Antibody titers reached their highest levels in May and June.

Öz (1999) reported that the seropositivity rate was 70.2% on the 17th day following vaccination, it reached 100% on the 75th day and then decreased to zero on the 189th day. He stated that antibodies detected against proplasm and schizont antigens were formed in all of the 57 vaccinated cattle (100%) and antibodies began to be detected from the 17th day and continued to be seen until the 189th day. The highest antibody titer was found to be 1:1280 on day 42 for schizont and 1:10240 on day 75 for piroplasm. The duration of seropositivity was at least 16.2 weeks for the 0-1 age group, and 19.3 weeks for the 1-2 year and the 2 year of age group.

Karatepe (2000) vaccinated seronegative cattle and found that 80.4% of them formed antibodies against *T. annulata* in the examinations made after 30 days. Seropositivity continued for 12 months, decreasing from the 6th month in the over 2-year of age group. In the group under two years of age, seropositivity started to decrease in the 4th month and ended in the 6th month. The antibody titer against the piroplasm antigen was determined between 1:20 and 1:2560, and the antibody titer against the schizont antigen was between 1:40 and 1:640. The highest antibody titers were detected in August and September against proplasm antigen and in June against schizont antigen.

Beniwal et al. (1997 and 2000) found that the immunity provided by the vaccine lasted for at least 6 months in the absence of infection in the field, and then the immunity began to weaken.

On the other hand, it is claimed that the effect of the live schizont cell culture vaccine developed against *T. annulata* to control the disease in China lasts for 19 months, and it is stated that cattle in enzootic regions exposed to infected tick infestations do not need to be vaccinated twice in their lifetime after being vaccinated once (Gu et al., 1997).

In this study, vaccination was done randomly, similar to the field application of the vaccine, which was not applied only to seronegative cattle as in other incidence studies. Therefore, as in the incidence studies mentioned above, a regular antibody course was not seen in this study, especially in serological examination with IFAT. However, at the first serological examination 1 month after vaccination, seropositivity increased from 0.4% to 88.9% in the group under 1 year of age and from 18.8% to 47.8% in the group over 1 year of age. Within 6 months, which is the effective period of the vaccine, seropositivity was found to be between 14.3-88.9% in the group under 1 year of age and between 22.5-77.8% in the group over 1 year of age. On the other hand, the first 6-month seropositivity rates in the control group were between 0.0-47.4% in Group 1 and 0.0-20% in Group 2.

In the second 6-month period, which is outside the effect of the vaccine, seropositivity was determined between 16.7-71.4% in the vaccinated Group 1 and between 47.1-77.1% in Group 2. In the control groups, seropositivity was found between 14.3-75.0% in Group 1 and 0.0-50.0% in Group 2 during this period.

According to the findings we obtained during the study, the seropositivity was 46.0% in the vaccinated group under 1 year of age and 29.1 in the control group cattle. Seropositivity was 55.6% in the vaccinated group over 1 year of age and 15.4% in the control group cattle. Since the vaccinated animals were randomly selected and blood samples were taken after vaccination, they could not be followed individually. In addition, since seropositive animals were not separated before vaccination, it could not be determined which antibody titers increased or decreased. However, an increase in seropositivity was noted following vaccination and did not reset for 1 year.

Such high seropositivity in the under-a-year-old control group can be explained by the fact that vector ticks were brought to the barns, possibly by cattle on pasture, and the disease was transmitted to them. The absence of death in these animals can be explained by the presence of maternal antibodies and the fact that the animals are crossbred and some of them are native breeds resistant to the disease.

In this study, antibody titers were determined using proplasm and schizont antigens. The antibody titer against proplasm antigen was found to be between 1:20-1:1280 in the vaccinated group under 1 year of age in the first 6 months, and between 1:20-1:640 in the group above 1 year of age. Likewise, it was observed that the antibody titer detected with piroplasm antigen in cattle under 1 year of age, was between 0-1:320 and 0-1:40 in the unvaccinated group above 1 year of age. In the second 6-month period, it was observed that the antibody titer was the same (1:20-1:320) in vaccinated cattle under 1 year of age and vaccinated over 1 year of age cattle. It was observed that it was between 1:20-1: 320 in the control group under 1 year of age and 0-1:160 in the control group over 1 year of age. Antibody titers detected with piroplasm antigen in vaccinated cattle were close to the results of Karatepe (2000) and lower than the others.

The antibody titers detected against the schizont antigen were found to be between 0-1:640 in cattle under 1 year of age and 1:40-1:640 in the group above 1 year of age in the vaccinated groups in the first 6 months. In the control groups, it was found between 0 - 1:320 in cattle under 1 year of age and 0-1:40 in the group above 1 year old. In the second 6-month period, the antibody titer detected with the schizont antigen was found to be between 0 - 1:160 in the vaccinated groups under 1 year old and between 0-1:640 in cattle over 1 year old. It was determined as 0 - 1:160 in cattle under 1 year of age in the control group and 0 (zero) in the group over 1 year of age. Antibody titers detected with schizont antigen were the same as those obtained by Karatepe (2000) in vaccinated cattle.

In the study area, cattle occasionally encounter infection. Therefore, instead of a gradual decrease in the antibody titer seen in other studies, occasional increases were detected. However, since the animals were vaccinated without distinguishing between seronegative and seropositive cattle in this study, it could not be determined exactly on which days the antibody titer formed and how long it lasted. However, it was determined that vector ticks were present in the region and infection prevailed. In addition, clinical theileriosis was detected in 1 cattle over 1 year of age and 2 cattle under 1 year of age. Sick animals were not treated and one of these vaccinated calves died. This was attributed to the excessively high parasitemia.

Conclusion

The fact that vaccination caused an increase in seropositivity and antibody titers in groups above and below 1 year of age cattle in the region indicates that vaccination activates the immune system. Again, serological and microscopic findings have shown that the region is stable endemic in terms of tropical theileriosis. Therefore, newly purchased cattle and newborn calves are at risk and should be vaccinated.

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