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ANAPLASMA MARGINALE INFECTIONS IN DAIRY CATTLE: CLINICAL DISEASE WITH HIGH SEROPREVALENCE

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Abstract

The fatal *Anaplasma marginale* infections were reported in a dairy cattle herd located in the Interior Aegean Region of Turkey. Fifteen Holstein dairy cows which had the symptoms of fever, weakness, depression, lack of appetite and decreasing of milk yields had died during January and March 2004. Inclusion bodies, characteristic of *A. marginale,* in erythrocytes of the sick cows were found. The presence of this agent was subsequently verified by serology. Then, the blood samples were collected from randomly selected 645 cattle which were grouped according to their age as follows: <9 months, 9-12 months, and 1-2, 2-3, 3-4, >4 years. The presence of *A. marginale* was determined by light microscopic examination of Giemsa-stained blood smears. Serum samples were tested for the presence of antibodies against *A. marginale* by cELISA. Of 645 animals, 357 (55.35%) and 220 (34.11%) were positive serologically and microscopically, respectively. The rates of the infection were different in the age groups. The specific antibodies against *A. marginale* were detected in 24.0%, 0.0%, 4.0%, 34.69%, 58.21%, and 82.07% of the cattle in the above age groups, respectively. The present report describes the first case of severe *A. marginale* infection in a cattle herd.

Key words: cattle, *Anaplasma marginale*, infection, symptoms, age, antibodies.

Anaplasma marginale is a ricketsial organism which causes a haemolytic disease in cattle in tropical and subtropical areas of the world. It is biologically transmitted by various tick species. Transmission may also occur mechanically by blood-feeding arthropods or by blood-contaminated fomites (13, 14, 16). Erythrocytes are the only known site of infection with *A. marginale* in cattle and more than 70% may become infected during acute infection. The symptoms of clinical disease are fever, anaemia, icterus, weight loss, abortion, lethargy and often death in animals older than 2 years (15, 22). *A. marginale* can usually be detected on Giemsa-stained thin blood smears in acute infections. Cattle that recover from acute anaplasmosis remain persistently infected with *A. marginale* (11). During the persistent infection, infected erythrocytes are not always detectable in stained blood smears (6, 17, 27), and thus, the diagnosis is usually made using a variety of serologic tests for the detection of specific antibodies (2, 5, 7). Recently, a competitive enzyme-linked immunosorbent assay (cELISA) was developed based on antibody binding to recombinant *A. marginale* major surface protein 5 (MSP5). This test was used to detect serologically both acute and chronic *Anaplasma* infections in cattle (12, 19, 21, 25).

Blood parasite infections are among the most important animal health problems for cattle industries in Turkey. Tropical theileriosis is widespread and most important tick-borne disease affecting, in particular, the very large numbers of the imported cattle. Babesiosis and anaplasmosis are also important. Anaplasmosis has been reported in almost every region of Turkey (8, 18, 20, 26). But it has a low incidence compared with theileriosis and babesiosis. The diagnosis of bovine anaplasmosis was primarily based on the microscopical blood smear examination. In a preliminary serological investigation on bovine anaplasmosis, 3.25% of cattle were positive for *A. marginale* antibodies by complement fixation test (4). Since then, other serological examinations have not been reported.

Although, severe economic losses have been reported due to anaplasmosis outbreaks in several parts of the world (3, 10, 13), no outbreak of *A. marginale* in cattle has been reported in Turkey before. The present report describes the first case of severe disease caused by *A. marginale* in a dairy cattle farm in the Interior Aegean Region of Turkey.

Material and Methods

Clinical history. The fatal infectious disease was observed in a herd of mostly dairy cattle located in the Interior Aegean Region of Turkey. The herd size was more than 1 200 animals. There were 370 dairy cows. The animals were housed in free range stables which were located adjacent to the stream. Fifteen dairy cows which had the symptoms of fever, weakness, depression, lack of appetite, and decreasing milk yields, died during January and March 2004.

Sample collection. The blood samples were collected from randomly selected 645 Holstein cattle which were grouped according to their age as follows: <9 months, 9-12 months, and 1-2, 2-3, 3-4, >4 years. The samples were obtained through jugular vein puncture. A thin blood film was prepared from each blood sample at the time of collection. The presence of *A. marginale* was determined by light microscopic examination of Giemsa-stained smears. Serum samples were also collected from the same animals, and were tested for the presence of antibodies against *A. marginale* by cELISA. The test was conducted in accordance with the method described by McElwain (17).

Statistical procedures. The results were analysed by *chi*-square test (24).

Results

The prevalence of *A. marginale* by cELISA and microscopic technique are shown in Table 1. Of 645 animals, 357 (55.35%) were positive for *A. marginale* antibodies. The results of serology and microscopy were not always concordant with each other. Two hundered and sixteen samples were positive by both tests, 284 samples were negative by both tests, 141 samples were serologically positive and microscopically negative, and 4 samples were serologically negative and microscopically positive.

The rates of the infection were different in the age groups. The specific antibodies against *A. marginale* were detected in 24.0%, 0.0%, 4.0%, 34.69%, 58.21%, and 82.07% of the cattle in age groups of <9 months, 9-12 months, and 1-2, 2-3, 3-4, >4 years, respectively (Table 2).

Anaplasma marginale inclusion bodies were found in 220 (34.11%) of 645 cattle. The organisms, of approximately 0.5-1.0 μ, were located peripherally in erythrocytes (Fig. 1).

Table 1
Comparison of cELISA with microscopic technique in the detection of *A. marginale*

Microscopy	cELISA		
	+	-	Total animals
+	216 (33.49)	4 (0.62)	220 (34.11)
-	141 (21.86)	284 (44.03)	425 (65.89)
Total animals	357 (55.35)	288 (44.65)	645

Table 2
The prevalence of *A. marginale* infection by cELISA in different age groups

Group	Age	Number of examined animals	Number of positive animals (%)	
			cELISA	Microscopy
I	<9 months	50	12 (24.00)[b]	5 (10.00)[b]
II	9-12 months	51	0 (0)[a]	0 (0.00)[a]
III	1-2 years	50	2 (4.00)[a]	2 (4.00)[ab]
IV	2-3 years	98	34 (34.69)[b]	24 (24.49)[c]
V	3-4 years	67	39 (58.21)[c]	34 (50.75)[d]
VI	>4 years	329	270 (82.07)[d]	155 (47.11)[d]
	Total	645	357 (55.35)	220 (34.11)

[a,b,c,d]: Different letters in the same columns are statistically significant ($P < 0.05$).

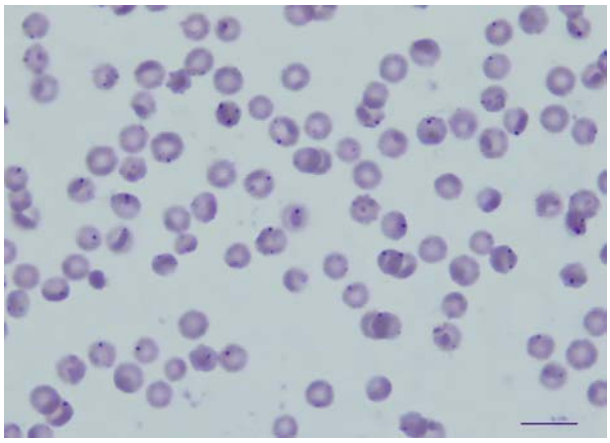

Fig. 1. Photomicrograph of a blood smear from *A. marginale*-infected cattle (Bar: 10 µm).

Discussion

Anaplasma marginale infection can be fatal in susceptible cattle and be partially responsible for the high rate of mortality observed in the affected herd (13, 23). The animals over 2 years of age are usually affected by a peracute fatal form of the disease (22). Calves from immune mothers receive temporary protection from the colostrum which prevents anaplasmosis. This protection lasts about 3 months, and in most cases, is followed by an age resistance, which lasts until the animals are about 9 to 12 months of age (13, 23). In the present study, it was observed that the clinical infections were significantly associated with age. The results of the general microscopic and serologic surveillance in the herd showed that cattle of all ages, except 9-12 months, is infected with *A. marginale*. However, the severity of illness and percentage of deaths increased with age. Fifteen dead animals were at the age of two years or above. Seropositive calves under 9 months of age did not exhibit any clinical signs of acute anaplasmosis. Our results are comparable to previous reports (13, 22, 23).

Calves exposed to anaplasmosis when the maternal or age resistance is high, rarely show clinical symptoms but develop a solid, long lasting immunity. It is therefore possible to have both *A. marginale* and cattle ticks present in a herd without animal loss or clinical disease. This situation is known as endemic stability (13, 14). The age resistance in calves gradually wanes after one year of age and these animals become increasingly susceptible to the disease in the regions which have not endemic stability (13, 23). In this study, the specific antibodies against *A. marginale* were detected in 24.0%, 0.0%, 4.0%, 34.69%, 58.21%, and 82.07% of the cattle in the age groups of <9 months, 9-12 months, and 1-2, 2-3, 3-4, >4 years, respectively (Table 2). Statistically significant differences were detected between the groups ($P < 0.05$). In the group of 9-12 months of age, no animals were seropositive. The rate of seropositive animals in the group of 1-2 years of age was only 4.0%. Consequently, the number of susceptible animals were higher in this group than in the other age groups. If susceptible adult cattle are mixed with infected cattle in the presence of the vectors, serious losses due to anaplasmosis can occur. More than 75% of calves did not show serological evidence of exposure to *A. marginale* in this study, and were not considered to have endemic stability for this organism. In the regions where the endemicity of the disease is instable, vaccination is an effective mean of preventing outbreak of anaplasmosis (13, 14). However, there is no vaccine against anaplasmosis in Turkey.

Cattle that recover from acute anaplasmosis remain persistently infected (the carriers of *A. marginale*). The carrier cattle are immune to reinfection, but serve as reservoirs of infection for transmission, and thus, contribute markedly to the spread of anaplasmosis (11, 13). In this farm, 55.35% of the animals were of the carrier status, and the remaining animals were susceptible to the infection. This position increases the risk of clinical disease in the future both in this and other farms. Control measures such as the gradual elimination of the *A. marginale* sero-reactors detected by serologic tests, the constitution of the distinct herds which consist of susceptible and carrier animals, and the elimination of carrier state by administration of antibiotics, are necessary, but it does not seem applicable in the herd.

During the persistent infection, infected erythrocytes are not always detectable in stained blood smears (6, 17, 27), and thus, the diagnosis is usually made using a variety of serologic tests for the detection of specific antibodies (2, 5, 7). Knowles *et al*. (12) reported that cELISA was positive in calves acutely infected with *A. marginale* before or concomitantly with the development of rickettsaemia, and that the antibodies were detectable in sera from persistently infected cattle inoculated as long as 6 years previously. In this study, the *Anaplasma* were not seen in the erythrocytes of 141 of 357 animals which were positive for *A. marginale* antibody by cELISA. The results show that cELISA is a favourable technique for the detection of acute and chronic anaplasmosis.

A. marginale can be transmitted biologically via ticks, while infected blood can also be transferred mechanically via fomites and biting insects (13, 14, 16). The tick control measures were not being applied in this farm because veterinary practitioners did not notice ticks or lice on animals. However, a few ticks may be able to transmit the infection (1). This outbreak probably occurred due to relaxed tick control measures. Furthermore, in the herd, rural practices such as bleeding, tagging, and vaccination are carried out frequently, therefore iatrogenic transmission is also possible (9). There is no information related with clinical anaplasmosis in other farms which are located near this farm. But, the likelihood of clinical disease for the susceptible animals is high in the future because the environment is suitable for the vector movements. The clinical infections may be prevented by using strict hygiene measures in rural practices and applying the acaricides periodically.

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