Prevalence of anaplasmosis on dairy farms in the European region (Thrace) of Türkiye: A retrospective study on the effectiveness of two different treatment protocols

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Received May 9, 2024
Accepted December 12, 2024

Abstract

This study was conducted in 2015–2022 to determine the status of anaplasmosis in breeding dairy cattle farms imported from abroad in the Thrace region of Türkiye. To investigate the presence of *Anaplasma* spp. blood samples with EDTA were taken from animals aged two years and older between 05.00 am and 10.00 am. Then a blood smear was prepared with the Diff-Quick staining method and examined under the light microscope at a × 100 magnification with immersion in oil. In addition, 40 randomly selected positive blood samples were analysed by the Nested PCR method. A total of 3,051 blood samples were examined by the Diff-Quick staining method. One hundred eleven of 966 samples (11.49%) in Kırklareli province and 297 of 2,085 (14.24%) samples in Tekirdag province were positive. As a result of nested PCR on 40 randomly selected positive samples from both provinces, the rates of *A. marginale*, *A. centrale*, and mixed infection (*A. marginale* and *A. centrale*) were determined as 34.5%, 4.0%, and 61.5% respectively. Of the 140 positive cattle, 70 were treated with oxytetracycline (Group 1), and the other 70 ones were treated with oxytetracycline-imidocarb combination (Group 2) twice with two month intervals for comparison. At the third examination, the rate of latent infection in the group treated with oxytetracycline-imidocarb combination was lower than in the group treated with oxytetracycline alone, but the difference was not significant ($P > 0.05$). As a result, it was revealed that anaplasmosis is a common disease in cattle in the Thrace region, and the combination of oxytetracycline and imidocarb was shown to be more successful than using oxytetracycline alone in reducing latent infected anaplasmosis cases.

Cattle, anaplasma, imidocarb, oxytetracycline

Anaplasmosis is a parasitic disease caused by obligate intracellular rickettsial agents that can cause disease in humans and many animals and is potentially endemic in 43 countries of the world (Constable et al. 2016; Atif et al. 2021). Anaplasmosis in cattle is caused by mainly intraerythrocytic rickettsial agents such as mainly *Anaplasma marginale*, *A. centrale*, *A. bovis,* and *A. ovis* and is of economic importance with its negative effects on productivity, health, and performance. Apart from them, *A. phagostophilum* which is a mononuclear agent, and *A. playtis* which is a thrombolytic agent can also cause bovine anaplasmosis (Kocan et al. 2010; Singh et al. 2015; Oğuz et al. 2018; Atif et al. 2021). While the inclusions in the erythrocyte in *A. marginale*, which is a more pathogenic species in bovine anaplasmosis, are mostly located at the edge of the erythrocyte, in *A. centrale*, which is less pathogenic than *A. marginale*, the inclusions are often located close to the center of the erythrocyte (Sevinç 2004; Kocan et al. 2010).

Bovine anaplasmosis is endemic in tropical and subtropical regions, the disease can be seen almost everywhere in the world, such as in Mexico, Central and South America, the Caribbean Islands, Europe, Mediterranean countries, Asia, Africa (Kocan et al. 2010; Aubry and Geale 2011; Constable et al. 2016; Atif et al. 2021). Although the disease can be seen in cattle in both beef (Coetzee et al. 2005) and dairy farms (Birdane et al. 2006),

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it is thought to be more common in dairy farms where there are more human manipulations such as routine vaccinations, diseases and treatments, insemination, blood transfusions, embryo transplants, and pregnancy controls (Gökçe et al. 2013; Constable et al. 2016). Again, it has been determined that the incidence of anaplasma infection may be higher in the Holstein breed than in the native breed (Tembue et al. 2011; Belal et al. 2014). The disease is mainly transmitted biologically by ticks, but it can also be transmitted mechanically by biting flies and blood-contaminated fomites. In addition anaplasmosis can be also transmitted vertically from infected mothers to the calves. The source of the disease may be infected ticks and reservoir animals (Sevinç 2004; Kocan et al. 2010; Aubry and Geale 2011; Constable et al. 2016). Nowadays, with global warming, the regional distribution of ticks, which are the biological vectors of the disease, is diversifying and the disease can be seen in wider geographies. Cattle are usually the main reservoir animal of bovine anaplasmosis but other domestic and wild ruminants such as buffalo and deer may also play a role in the transmission of the disease. Again, contact with wild animals, use of common pastures and increased settlement in forest/rural areas increase the incidence of the disease (Kocan et al. 2010; Aubry and Geale 2011; Marquez et al. 2020).

Anaplasma marginale is the main pathogenic species in bovine anaplasmosis, therefore, much research has been focused on and limited to it. The seroprevalence of *A. marginale* has been reported as 13.04% in Iraq (Jassem and Agaar 2015), 31.05% in Pakistan (Atif et al. 2013), 50.2% in Egypt (Al-Hosary et al. 2020), 67.1% in Mexico (Ferreira et al. 2022), 76.5% in Mozambique (Tembue et al. 2011), 80.8% in Hungary (Hornok et al. 2007) and 36.6% in Brazil (Ferreira et al. 2022). Although there is no clear information on *A. marginale* seroprevalence in cattle in Greece and Bulgaria, which are adjacent to the Thrace region, it has been determined that 20.1% of ticks in Greece are positive for *Anaplasma* spp. (Efstratiou et al. 2021). In another study, the seroprevalence of *A. ovis* in cattle in Greece was stated as 35.9% (Giadinis et al. 2015). There are many regional-based studies on *A. marginale* seroprevalence in Türkiye which is a third neighboring country part (Türkiye, Greece, and Bulgaria) of Thrace. In this sense, the seroprevalence of *A. marginale* is detected as 28.6%–52.1% in the eastern Anatolia and southeastern Anatolia regions (Van, Muş, Siirt, Diyarbakır, Kars) (Gökçe et al. 2013; Oğuz et al. 2018), 55.35% in the Aegean region (Birdane et al. 2006), 37.8% in the Black Sea region (Acıcı et al. 2016), and 31.86% in the Central Anatolia region (Konya) (Işık et al. 2018).

Anaplasmosis in cattle can be in peracute, acute, subclinical, and latent forms. In acutely infected cases, the main clinical findings are high fever, weakness, anorexia, increased respiratory and heart rate, tachypnoea, anaemia of varying severity, lacrimation, decrease in milk yield, abortion, temporary infertility, and sometimes death (Kocan et al. 2010; Singh et al. 2015; Shane et al. 2020). Cattle that recover from acute infection develop persistent/latent infections characterized by cyclic low-level rickettsaemia (Kieser et al. 1990; Aubry and Geale 2011; Costable et al. 2016).

The diagnosis of the disease can be made with epidemiological data (being in areas where the disease is endemic, seasonal tick activity, etc.), relevant clinical findings, and necropsy findings (Kocan et al. 2010; Constable et al. 2016). However, microscopic examination of blood smears or competitive ELISA a serological test, or PCR-based molecular tests are used to confirm the diagnosis. To ensure definitive identification on a species basis of the rickettsial agents detected in the microscopic examination, PCR-based molecular tests are used (Kocan et al. 2010; Aubry and Geale 2011; Öter et al. 2015; Atif et al. 2021).

It is known that from the past to the present, tetracycline group antibiotics such as oxytetracycline (Coetzee et al. 2005; Atif et al. 2012; Alberton et al. 2015; Singh et al. 2015), chlortetracycline (Curtis et al. 2021; Toillion et al. 2021) and doxycycline (Kuttler and Simpson 1978) have been the most frequently used medicals in the treatment of bovine anaplasmosis. Apart from this, it has been reported that enrofloxacin

(Coetzee and Apley 2006; Facury-Filho et al. 2012; Alberton et al. 2015; Singh et al. 2015; Shane et al. 2020), imidocarb dipropionate (Atif et al. 2012; Alberton et al. 2015), diminazene aceturate (Alsaad 2007) and even arsenic, antimalarials, and antimony derivatives are also used in the treatment of anaplasmosis in cattle (Kocan et al. 2010; Aubry and Geale 2011). As none of these known treatment choices are 100% successful in eliminating persistent infections or eliminating carrier animals, more effective, and successful alternative treatment quests are continued (Kocan et al. 2010; Alberton et al. 2015; Curtis et al. 2021).

This retrospective study aimed to determine the prevalence of anaplasmosis between 2015 and 2022 on farms with dairy cattle imported from abroad, that are located in the Thrace region of Türkiye, and to compare the effectiveness of two different treatment regimens in *Anaplasma* spp. positive animals.

Materials and Methods

This study was carried out with the approval of the Bursa Uludag University Animal Experiments Local Ethics Committee (2024–07/02). The material of the presented retrospective study consisted of a total of 3,051 blood samples taken from enterprises with dairy cattle imported from abroad, located in the Thrace region of Türkiye (two provinces on the border between the European part, Bulgaria, and Greece) between 2015 and 2022. Blood samples were provided from four farms in each province, 966 from Kırklareli (41°44'-42°00'N, 26°53'-41°44'E) and 2,085 from Tekirdağ (40°36'-41°31'N, 26°43'-28°08'E), respectively.

In the study, blood samples were taken to the tubes containing EDTA from Holstein cattle aged two years and above in each farm between 05.00 and 10.00 a.m., and blood smears were made. Blood smears were prepared by the Diff-Quick staining method, and then examined by a light microscope with a × 100 magnification for the presence of *Anaplasma* spp. In addition, 40 samples were randomly selected from *Anaplasma* spp. positive in the microscopic examination and were examined in Nested PCR (Lew et al. 2002; Aktas and Özübek 2017). Also, a necropsy was performed and examined in animals that died due to anaplasmosis in the enterprises during the research period.

A total of 140 cattle, which were found to be *Anaplasma* spp. positive in microscopic examination, were divided into two equal groups, and the following treatment was applied.

Group 1: Oxytetracycline (Primamycin LA®, Zoetis, Parsippany, NJ, USA) was administered to 70 cattle in this group, intramuscularly at a dose of 20 mg/kg × 3 with an interval of 48 h and two months later the same treatment protocol was repeated (20 mg/kg × 3 with an interval of 48 h).

Group 2: Oxytetracycline (Primamycin LA®, Zoetis) was applied to 70 cattle in this group in the same way as in Group 1 (at a dose of 20 mg/kg, × 3 at 48-h intervals, and repeated 2 months later). In this group, also imidocarb dipropionate (Imicarp®, Teknovet, Istanbul, Türkiye) was administered intramuscularly at a dose of 3 mg/kg twice at two-month intervals.

In both groups, blood samples were taken two months after the first and second treatments, and the latent infection levels of the groups were compared by microscopic examination. Statistical evaluations were made using the SigmaPlot® statistical package program, using Pearson's Chi-square test for comparisons of latent infected cattle percentage (rickettsiaemia positive ones) between different treatment groups. In all evaluations, $P < 0.05$ was considered significant.

Results

In the study, 111 of 966 samples (11.49%) taken from Kırklareli province and 297 of 2,085 samples (14.24%) in Tekirdağ province were found to be *Anaplasm*a spp. positive in microscopic examination of the blood smears. In the microscopic examination, *A. marginale* and *A. centrale* were assigned as positive if the rickettsial agents were at the periphery of the erythrocytes, and were in the centre of the erythrocytes, respectively (Plate I, Fig. 1).

The annual number of samples examined microscopically, the number of *Anaplasma* spp. positive cases, and their percentages in both provinces during the research period (2015–2022) are summarized in Table 1. By examining 40 randomly selected *Anaplasma* spp. positive samples from both provinces by nested PCR, the rates of *A. marginale, A. centrale*, and mixed infection (*A. marginale* and *A. centrale*) were determined as 34.5%, 4.0%, and 61.5%, respectively.

During the study, 16 cattle died of anaplasmosis. In the diseased animals clinical findings were initially observed such as high fever, anaemia, and jaundice. At necropsy, varying degrees of jaundice, splenomegaly, petechial haemorrhages in the intestines and apparent pericarditis in some were found (Plate I, Fig. 2).

The changes in parasitaemia levels in microscopic examination after treatments applied at two-month intervals in anaplasmosis positive cattle are presented in Table 2. When evaluating the parasitaemia levels in Group 1 and Group 2, they were detected as 25.7% and 24.7%, respectively ($P = 0.85$), after the first treatment regimen, and 12.5% and 8.57%, respectively ($P = 0.41$), after the second treatment.

Table 1. The number and rates of *Anaplasma* spp. positive cattle by years in microscopic examination of blood smears in both provinces.

		Years								
Province		2015	2016	2017	2018	2019	2020	2021	2022	Total
Kırklareli	Sample numbers	110	114	106	122	112	106	109	187	966
	Positive n	14	12	9	13	9	8	17	29	111
	Positive %	12.72%	10.52%	8.50%	10.65%	8.03%	7.54%	15.59%	15.50%	11.49%
Tekirdağ	Sample numbers	192	282	291	278	266	198	238	340	2,085
	Positive n	32	39	41	42	27	28	34	54	297
	Positive %	16.66%	13.82%	14.01%	15.10%	10.15%	14.14%	14.28%	15.88%	14.24%
Total	Sample numbers	302	396	397	400	378	304	347	527	3,051
	Positive n	46	51	50	55	36	36	51	83	408
	Positive %	15.23%	12.87%	12.59%	13.75%	9.52%	11.84%	14.69%	15.74%	13.37%

Table 2. Number and percentages of cattle with ongoing rickettsaemia after treatments in the groups.

Treatment group	Positive animals before treatment protocol	Positive animals after 2 months from the1st treatment protocol	Positive animals after 2 months from the 2nd treatment protocol
Group 1 (Oxyt)	70 (100%)	18 (25.7%)	9 (12.85%)
Group 2 (Oxyt + Im)	70 (100%)	17 (24.7%)	6 (8.57%)

Oxyt: oxytetracycline administration; Oxyt + Im: oxytetracycline and imidocarb dipropionate administration

Discussion

Although it has been reported that ticks generally play a primary role in the spread of *Anaplasma* spp. in nature (Kocan et al. 2010; Aubry and Geale 2011; Atif et al. 2021), the fact that the disease was seen on intensive dairy farms without the presence of ticks in the presented study showed that mechanical transmission is essential in anaplasmosis. In this sense, it has been revealed that the high prevalence of anaplasmosis in enterprises is caused by mechanical contamination with flies and iatrogenic errors such as injection and blood collection.

In a previous study conducted with molecular analyses in cattle in Bartın province in Türkiye, *A. marginale* (18.8%) and *A. centrale* (18.0%) were detected at almost the same density (Aktaş and Özübek 2015). The fact that *A. marginale* (34.5%), which is a much more pathogenic agent, was found to be × 8.6 more common than *A. centrale* (4.0%) in the present study revealed the regional difference as well as the higher risk in the Thrace region. On the other hand, when the anaplasma positive cattle were evaluated

in the eight-year research period, it was seen that the positive animal rates varied between 9.52% and 15.74%. Although the rates of positive cases were generally similar over the years in this retrospective study, the increase in the positive case rate which was found to be 9.52% in 2019, after 2020 was attributed to the constant import of cattle to the farms. Although there has been no previous study on the prevalence of anaplasmosis in cattle in the Thrace region, the rates of *A. ovis*, *A. phagocytophilum*, and mixed infection (*A. ovis* and *A. phagocytophilum*) in small ruminants were determined as 50.83%, 8.51%, and 4.96%, respectively (Öter et al. 2015).

Many drugs have been tried in the treatment of anaplasmosis from the past to the present and different results have been obtained as a result of the treatment. It is noteworthy that the most commonly used medical group in treatment of anaplasmosis is oxytetracyclines. Studies on the use of oxytetracycline in bovine anaplasmosis show that it is used in different forms (long/short effect) and for many different periods (Coetzee et al. 2005; Atif et al. 2012; Facury-Filho et al. 2012; Alberton et al. 2015; Singh et al. 2015). In this sense, it is known that oxytetracycline is used at a dose of 10 mg/kg once a day for five days (Singh et al. 2015), at a dose of 30 mg/kg as a single dose (Coetzee et al. 2005), and at a dose of 30 mg/kg twice with an interval of five days (Coetzee et al. 2005). Apart from this, long-acting oxytetracycline has been reported to be used as a single dose at a dose of 20 mg/kg (Facury-Filho et al. 2012), once a day at a dose of 22 mg/kg for five days (Coetzee et al. 2005; Atif et al. 2012), and at a dose of 20 mg/kg × 3 with an interval of 48 h (Alberton et al. 2015).

Imidocarb is another popular drug for the treatment of bovine anaplasmosis, and it has been generally used a single or repeated (twice every week) dose of 2.5–5 mg/kg (Akhter et al. 2010; Atif et al. 2012; Alberton et al. 2015). Akhter et al. (2010) reported that two intramuscular injections of imidocarb at a dose of 3 mg/kg could eliminate the *Anaplasma* spp. On the other hand, Alberton et al. (2015) reported that a single dose of 2.5 mg/kg imidocarb made only 53.5% of the cases negative. Al Saad (2007) reported that imidocarb at a dose of 3.5 mg/kg repeated every 48 h was superior to the treatment effectiveness against anaplasmosis compared to oxytetracycline at a dose of 20 mg/kg repeated every 48 h. Also, Roby and Mazzola (1972) reported that chemosterilization was achieved by applying imidocarb at 5 mg/kg twice with an interval of 14 days. Shane et al. (2020) found that the administration of a single dose of 12 mg/kg enrofloxacin to cattle with acute infection with *A. marginale* improved clinical scoring by 81.1% and reduced the mortality rate from 47% to 3% at the end of the 28th day.

Facury-Filho et al. (2012) stated that enrofloxacin and oxytetracycline both have positive effects in treatment, but enrofloxacin provides a faster effect in reducing rickettsaemia and normalizing haematocrit. Another study on the clinical effectiveness of imidocarb dipropionate, enrofloxacin, and oxytetracycline in cattle infected with *A. marginale* determined that although the use of enrofloxacin was more effective, all three drugs caused a decrease in the rickettsaemia level throughout the treatment, however, neither protocol could completely eliminate the disease after the end of the treatment (Alberton et al. 2015). The differences in the results of different studies have been interpreted as varying depending on factors such as the dose and method of use of the drugs, differences of the *Anaplasma* spp. strain, differences in the analysis method, drug resistance, etc. As a matter of fact, many different strains of *A. marginale* that have been identified worldwide differ in genotype, antigenic composition, morphology, and infectivity for ticks. Again, the high resistance to antimicrobials especially oxytetracyclines almost everywhere today also reduces the success of treatment (Kocan et al. 2010; Aubry and Geale 2011). It has been reported that for oxytetracycline to be effective on anaplasmosis, it must reach an antibiotic concentration in an appropriate time and above the minimum inhibitory concentration (Alberton et al. 2015). In this sense, the World Organisation for Animal

Health (WOAH) recommends the intravenous administration of oxytetracycline at a dose of 22 mg/kg for five days for the treatment of anaplasmosis in cattle (Coetzee et al. 2005; Kocan et al. 2010), but it was stated in some studies that this treatment was insufficient to treat persistent infections (Coetzee et al. 2005; Curtis et al. 2021).

Since there is no report in the literature on the simultaneous use of oxytetracycline and imidocarb combined in the treatment of bovine anaplasmosis, such combination is used in bovine anaplasmosis for the first time, and compared for treatment effectiveness with the oxytetracycline only protocol in the present study.

Atıf et al. (2012) reported that oxytetracycline and imidocarb improved 86.67% and 73.33% of cases, respectively, in cattle infected with *Anaplasma* spp. in the second month after treatment. It was found in the presented study (with two treatment cycles with two-month intervals) that 87.15% of carrier cases improved with the use of oxytetracycline alone, and 91.43% of carrier cases improved with the combination of imidocarb and oxytetracycline. Thus, it was concluded that although there was no significant difference ($P > 0.05$), the combined use of oxytetracycline and imidocarb was superior in reducing parasitaemia than the use of these drugs alone.

In conclusion, it was observed that anaplasmosis is a common disease on dairy farms located in the Thrace region with cattle imported from abroad. The high incidence of anaplasmosis in our country and border region makes it necessary to take protective measures. In this sense, it is recommended that screening tests, which are limited to notifiable diseases in animal import policies, should also be carried out against latent diseases such as anaplasmosis. Again, the fact that ticks were not present on the farms during or before the study suggested that anaplasmosis may be caused by inadequate fly control or common injector needles, so the importance of paying attention to these issues in the control of the disease needs to be emphasized.

It has been found that the use of oxytetracycline and imidocarb dipropionate combined instead of oxytetracycline alone reduces the latent infection rate in the treatment and control of the disease. Thus, combined treatment can be useful in providing an advantage for the control of the disease on high risk farms.

Considering the prevention of the spread of the disease which is also important from the economic point of view, it is recommended to exliminate those animals that are still found positive on microscopic examination (latent ones) from the herd after the second treatment despite the use of combined drugs.

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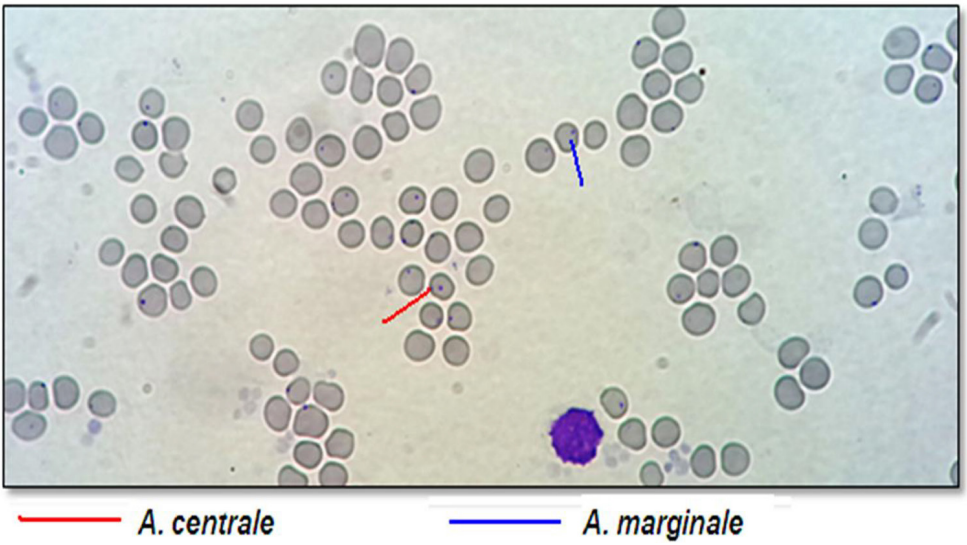

Fig. 1. *Anaplasma centrale* and *A. marginale* inclusions in blood smear microscopy

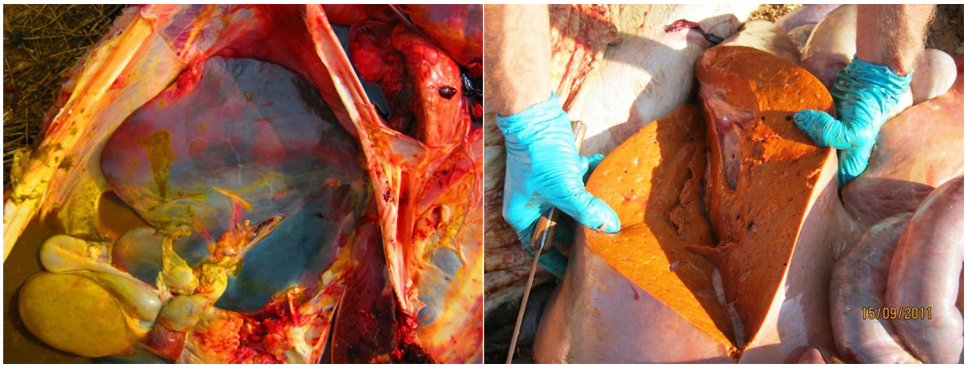

Fig. 2. Severe icterus of the internal organs, especially the liver and omentum, in affected cattle