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Seropositivity of *Mycobacterium paratuberculosis* in Cattle with Chronic Diarrhea in the Middle Black Sea Region

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Absract: Paratuberculosis (Johne's disease), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is one of the most common and contagious infections of farm animals mainly cattle herds. MAP leads to a chronic and progressive enteric disease that results poor body condition, reduced milk production, premature culling, and decimate slaughter value in cattle industry. The aim of this study was to investigate of the seropositivity of MAP in cattle with chronic diarrhea in Middle Black Sea Region. For this purpose, a total of 859 Holstein-Friesian dairy cattle (>2 years old) from 15 herds with companied to chronic diarrhea were examined in Amasya and Samsun provinces, between 2012 and 2014. MAP antibodies were determined by indirect commercial enzyme-linked immunosorbent assay ELISA (iELISA) kit. Of the 859 samples, 86 (10.0%) and of 15 herds, 7 (46.7%) were positive for the MAP antibodies. In 2012, 2013 and 2014, positive rates of 2.4%, 21.5% and 50% were detected, respectively. As a result, there was a significant increase in MAP seropositivity in chronic diarrheal cattle. It is thought that the results of the study will contribute to the development of effective control programs for eradication of the disease and clinicians working in the region will benefit from the attention of veterinarians.

Keywords: Cattle, İELISA, Diarrhea, Johne's disease, Paratuberculosis.

Orta Karadeniz Bölgesinde Kronik İshalli Sığırlarda *Mycobacterium paratuberculosis'in* Seropozitifliği

Öz: Paratüberküloz (Johne's hastalığı), *Mycobacterium avium* subsp. *paratuberculosis* (MAP) tarafından oluşturulan, başta sığır sürüleri olmak üzere çiftlik hayvanlarının en yaygın ve bulaşıcı enfeksiyonlarından birisidir. MAP sığırlarda kronik ve ilerleyici bir enterik hastalığa neden olarak vücut kondüsyon kaybı, düşük süt verimi, erken kesim ve düşük kesim değerine neden olan bir hastalıktır. Bu çalışmada, orta Karadeniz bölgesindeki kronik ishalli şikayeti olan sığırlarda MAP antikor prevalansının tespiti amaçlandı. Bu amaçla 2012-2014 yılları arasında Amasya ve Samsun illerindeki 15 işletmeden 2 yaş ve üzeri kronik ishalli 859 Holstein-Friesian süt sığırı incelendi. MAP antikorları ticari bir indirekt ELISA kiti (iELISA) ile belirlendi. İncelenen serum örneklerinin 86'sı (%10,0) ve işletmelerin 7'si (%46,7) MAP antikorları yönünden pozitif olarak bulundu. 2012, 2013 ve 2014 yıllarında sırasıyla %2,4, %21,5 ve %50,0 oranında pozitiflik saptandı. Sonuç olarak, kronik ishalli sığırlarda MAP seropozitifliği bakımından belirgin bir artış olduğu görüldü. Çalışma sonuçlarının bölgede görev yapan klinisyen veteriner hekimlerin dikkatini çekmek açısından faydalı olacağı ve hastalığın eredikasyonu için etkin kontrol programlarının geliştirilmesi yönünde önemli katkılar yapacağı düşünülmektedir.

Anahtar Kelimeler: iELISA, İshal, Johne's hastalığı, Paratüberküloz, Sığır.

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INTRODUCTION

Paratuberculosis, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is a chronic, granulomatous enteric disease in cattle with worldwide distribution having a significant impact on the world economy that does not respond to treatment. The most critical affecting dairy cattle around the world showing symptoms of an insidious intestinal pathology responsible for significant economic losses. These losses are mainly related with reduced milk production, veterinarian costs, premature culling, and reduced slaughter value (1,2). MAP generally gets into the herds by purchasing of the infected cattle that appear clinically normal. The movement of contaminated vehicles or equipment between herds is also another route by which the disease can be introduced (3). MAP could be stay alive more than a year in the feces of cattle and around 160 days within the surface water. Younger animals are the most susceptible to MAP infection, especially shortly after birth, although clinical signs typically do not appear for 3–5 years reflecting the long incubation period and slow course of the disease (2,3).

MAP cases are encountered across the continents; its regional and territorial distributions show differences. The prevalence of MAP infection in cows is demonstrated to be between 3-30% in European countries (1) and between 4.6-20% in Turkey, as well (4-6). However, to the authors' knowledge there are any reports on the prevalence of the disease in cattle companied with chronic diarrhea. In this study, it was aimed to determine the seroprevalence of paratuberculosis in dairy cattle with chronic diarrhea in Middle Black Sea Region.

MATERIALS and METHODS

Animals and Sampling

In total, 859 blood samples were taken from Holstein-Friesian dairy cattle (>2 years old) with complained of chronic diarrhea between 2012 and 2014. Seropositive samples were taken in the following years. The samples were collected from animals by authorised veterinarians during clinical examinations following standard procedures. A total of 9 ml of blood was collected from the jugular vein of each animal in a vacutainer with serum clot activator. Separated serum samples were stored at -20°C until used. Blood samples were taken from 15 dairy farms in Amasya and Samsun provinces (in 7 different towns) located in Middle Black Sea Region of Turkey. Sampled dairy farms had 57-1350 cows and approximately 5.7-13.5% blood samples were taken per farm. The main criteria for the selection of these farms are larger than fifty dairy cattle, history of chronic diarrhea which is not responding to classical diarrhea treatment, and with no history of vaccination against MAP over two consecutive years. In this area, there are approximately 400.000 cattle; however, there are 128 farms which are more than 50 dairy cattle according to the Ministry of Food, Agricultural and livestock data. Simple purposive sampling was conducted with the herd as the epidemiological unit of concern. The sample size was calculated as three hundred and eighty-four within a confidence level of 95% and confidence interval (CI) of 5% and considering the cattle number of Middle Black Sea Region. The study was conducted in accordance with the ethical principles for animal experiments.

Sample Analysis

A commercial indirect enzyme-linked immunosorbent assay (i-ELISA) kit (MAP Ab-ELISA Test Kit, IDEXX Laboratories, Inc., Westbrook, Maine) for detection of antibodies against MAP was used and all samples were measured by an ELISA reader (Mindray MR-96A). Test plates were measured in 450 nm filter at the end of the test and determined OD values were calculated. The test procedure and interpretation of the results were made through out

according to the manufacturer's instructions. Results were expressed as a sample to positive (S/P) ratio after correction with the negative control. A sample was expressed as positive in case of S/P ratios equal to or greater than 55%.

RESULTS

The results of serological examination by i-ELISA are shown in Table 1. Serum samples were analyzed and 86/859 (10.0%) and 7/15 of the herds (46.7%) were seropositive for the MAP antibodies. The three-year study period covering 2012, 2013 and 2014 revealed MAP seropositivity as 2.4%, 21.5% and 50%, respectively. With respect to herd seropositivity MAP antibody for the years 2012, 2013 and 2014, the seropositvity was 2/6 (33.3%), 2/4 (50%), and 3/5 (60.0), respectively, in total making up 7/15 (46.6%). However, two herds from Samsun region were negative in the year 2012 while there was one positive herd (38.1%) from same region in the year 2013.

Table 1 Seropositivity distribution of MAP by origin and years.
Tablo 1 MAP seropozitifliğinin orijin ve yıllara göre dağılımı.

Origin	2012 (%)	2013 (%)	2014 (%)	Total (%)
Amasya	15/558 (2.7)	23/123 (18.7)	40/80 (50.0)	78/761 (10.2)
Samsun	0/77 (-)	8/21 (38.1)	-	8/98 (8.1)
Total	15/635 (2.4)	31/144 (21.5)	40/80 (50.0)	86/859 (10.0)

DISCUSSION and CONCLUSSION

Johne's disease caused by MAP is one of the most prevalent and costly infectious diseases of the cattle practice. Johne's disease has widespread worldwide importance. As a result, multiple studies have been carried out to determine the within-herd and between-herd prevalence of MAP infections (1). The serological tests commonly used for paratuberculosis in cattle are ELISA, complement fixation (CF) and agar gel immunodiffusion (AGID). The ELISA is the current method that used in the serological diagnosis of paratuberculosis, and can be conducted rapidly with results in reliable data and requires only limited expertise (7-9).

Paratuberculosis is widespread in all ruminant populations in almost all countries with the dairy cattle. Seroprevalence of paratuberculosis worldwide varies from 15% to 78%. The prevalence of paratuberculosis in European countries has been reported to vary between 0% and 75% (1). Böttcher and Gangl (10), reported the infection prevalence as 42.0% in Germany. On the other hand, studies performed on dairy cattle in different countries indicate that the prevalence of infection in the herd is similar and reported to be 55% in Denmark and 68% in France (1), 43% in Canada (11), 50% in the United States (12), 0.9/18% in Belgium (13), 42% in Italy (14), 31.5% in Ireland (15), 54% in the Netherlands (16), 17.0% in United Kingdom (17). Similarly, in this study the herds' prevalence was detected as 46.7% for the MAP antibodies. With respect to herd seropositivity MAP antibody for the years of 2012, 2013, and 2014 the seropositivity was 33.3%, 50.0%, and 60.0%, respectively. Our research data show remarkably high level of cattle seropositivity in the three years wise reaching 2.4%, 21.5% and 50%. The most important reason for the increase is the repeat samples from seropositive farms. Because seropositive samples were taken in the following years. Seroprevalence difference of paratuberculosis between countries and provinces may be due to climate and geographical conditions, nutrition and housing conditions, the number of animals in farms, pasture, and vaccination status (1). In this study, the study area (Amasya and Samsun) is in Middle Black Sea Region and the climate of this region with high and evenly distributed rainfall the year round. The increase in seropositivity rates may be due to the availability of climatic conditions and inadequate herd hygiene conditions. Moreover, sampled farms were intensive farm unite and unvaccinated against MAP.

The presence of paratuberculosis has been known throughout Turkey (4,5,6). However, the numbers of the studies inspecting the infection and its prevalence are limited. Moreover, there is no scientific report on the prevalence of the disease in dairy cattle with chronic diarrhea. Prevalence studies of paratuberculosis carried out from different regions of Turkey revealed to be between 4.6-20% by different diagnostic tests (4,5,18). Vural and Atala (19) reported the prevalence of the infection in the Central Anatolia Region as 4.6% by ELISA. Seropositivity was reported between 6.2 to 58.3% in Burdur province (20). The seroprevalence of subclinical paratuberculosis was 3.5% and farm prevalence was 41.6% in Kars district (4). Seropositivity rates were detected between 4 to 20% in Usak province (5), and 3.4-5% in Elazig province (21). In the present study, seropositivity was detected as 10% (86/859). Herd/true seropositivity rates determined in this study were similar to the results compared with the data obtained from the different regions of Turkey and other countries (4,11,12,20).

In conclusion, paratuberculosis is detected increasing by years with a high prevalence in cattle with chronic diarrhea in Middle Black Sea Region. It is thought that the results of the study will contribute to the development of effective control programs for eradication of the disease and clinicians working in the region will benefit from the attention of veterinarians.

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