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RESEARCH ARTICLE

Seroprevalence of Subclinical Paratuberculosis in Dairy Cattle in Manisa Region of Turkey

Mehmet BERBEROĞLU[1], Turan CİVELEK[1]*

[1]*Department of Internal Medicine, Faculty of Veterinary Medicine, University of Afyon Kocatepe, Afyonkarahisar, TÜRKİYE*

Corresponding author e-mail: tcivelek@aku.edu.tr

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ABSTRACT

The purpose of the study was to determine the seroprevalence of subclinical paratuberculosis in dairy cattle in Manisa region of Turkey. Within this research, sampling was performed in a total of six districts including the central district of Manisa and 40 different cowsheds. The study material consisted of 442 clinically healthy multiparous Holstein dairy cattle. Live weights, the previous lactation milk yield, and age of the animals were recorded. Seroprevalence was determined by ELISA in the collected sera samples. The seroprevalence of the disease was found to be 13% in the central district of Manisa, 28% in Turgutlu, 20% in Alasehir, 38% in Kula, 26% in Akhisar, and 22% in Salihli. Infection was determined in all samples collected from all cowsheds. The results of the study revealed that seroprevalence of subclinical paratuberculosis in dairy cattle in Manisa region of Turkey was 21.72%.

Keywords: Johne's disease, Manisa region, Paratuberculosis, Prevalance.

Manisa Yöresi Süt Sığırlarında Subklinik Paratüberkülozun Seroprevalansı

ÖZ

Bu çalışmanın amacı Manisa yöresi süt sığırlarında subklinik paratüberküloz seroprevalansını belirlemektir. Araştırma kapsamında, Manisa merkez ilçe dahil olmak üzere altı ilçede ve toplam 40 farklı ahırdan örnekleme gerçekleştirildi. Çalışma materyalini klinik olarak sağlıklı 442 multiparoz Holstein ırkı süt sığırı oluşturdu. Hayvanların canlı ağırlıkları, önceki laktasyon süt verimleri ve yaşları kaydedilmiştir. Seroprevelans, toplanan serum örneklerinde, ELISA metodu ile belirlendi. Hastalığın seroprevalansı Manisa merkez ilçede %13, Turgutlu'da %28, Alaşehir'de %20, Kula'da %38, Akhisar'da %26, ve Salihli'de %22 olarak belirlendi. Örnekleme yapılan tüm ahırlarda enfeksiyon varlığı tespit edilmiştir. Çalışma sonuçları, Manisa yöresi süt sığırlarında subklinik paratüberküloz seroprevalansının %21.72 olduğunu ortaya koydu.

Anahtar Kelimeler: Johne's Hastalığı,Manisa Bölgesi, Paratüberküloz, Prevalans.

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INTRODUCTION

Paratuberculosis is an infectious disease induced by *Mycobacterium avium subsp. paratuberculosis* (MAP). It causes chronic diarrhea in ruminants. This disease is reported to be in zoonotic character in humans due to its role in the etiology of Crohn's disease (Nakase et al. 2006, Diequez et al. 2009).

Paratuberculosis (pTB) is subclinical in the infected cattle for a long time. In many cattle, the factor cannot be detected in fecal samples before the age of two (Andrews 2004, Diequez et al. 2009). At this stage, the infected cattle continue to contaminate the healthy ones with the factor (Baumgartner and Khol 2006). The infected animals not showing clinical symptoms as yet may start to disseminate the factor through their fecal matters about 15-18 months before the emergence of active infection (Smith and Bradford 2001). At this early stage of infection, clinical symptoms are not observed in ruminants (Allaker and Kapas 2003, Baumgartner and Khol 2006). With the progress of the disease, MAP spreads to regional lymph nodes and clinical symptoms arise. Typical clinical manifestations are chronic diarrhea and weight loss (Andrews 2004). pTB causes yield losses in infected animals. It is one of the most important diseases causing economic losses in dairy cattle enterprises (Collins et al. 1994). Studies on pTB have been conducted in Turkey and the issue keeps its actuality (Civelek et al. 2009, Makav and Gokce 2013, Ozturk et al. 2010, Yıldırım and Civelek 2013). Current studies have generally been conducted to demonstrate the regional prevalence of the disease (Cetinkaya et al. 1999). However, the nation-wide prevalence of the disease in Turkey could not be fully determined. Subsequently, the impacts caused by this disease are not known clearly (Mecitoglu and Demir 2012).

Regional and national prevalence of MAP varies. In many countries in the world, with different prevalence rates, the presence of pTB has been reported (Collins et al. 1994, Jakobsen et al. 2000, Hacker et al. 2004).

Clinical diagnosis of paratuberculosis is usually made on the grounds of symptoms, anamnesis, and necropsy results. Diagnostic laboratory tests are utilized in patients showing no symptoms. There is not any available test with high specificity and sensitivity which can particularly be used for young cattle (Baumgartner and Khol 2006). However, different methods can be used in the diagnosis of pTB. MAP can be diagnosed by mainly utilizing culture, PCR, immunity tests, and serological tests (Muskends et al. 2003, Baumgartner and Khol 2006, Civelek et al. 2009, Mecitoglu and Demir 2012).

Thanks to serological tests, the presence of MAP is evaluated swiftly, and the diagnosis can be made (Stricklands et al. 2005). ELISA allows the identification of antibodies against MAP. Today, it is the most commonly used method in the diagnosis of the disease (Kalis et al. 1999, Jubb et al. 2004, Yıldırım and Civelek 2013). It is an extremely easy-to-apply test with fast sample analyses. It is usually used for testing serum samples (Collins et al. 2005). ELISA is the most affordable and easily applicable method to be used in herd paratuberculosis control programs. In the field studies conducted by ELISA test, 80% of the infected animals have been diagnosed before showing clinical symptoms. It was also reported that 65% of the animals not contaminating the environment with the factor through their feces yet were determined in advance (Jubb et al. 2004). ELISA is the most reliable method of determining subclinically infected animals (Stricklands et al. 2005).

There is no effective treatment of pTB, under the present circumstances. In order for disease prevention and control, the animals diagnosed as MAP-positive must be eliminated from the herd. In MAP-negative herds, necessary precautions must be taken to prevent a possible infection. In MAP-suspicious herds, the investigation of factor presence by serological tests applied at intervals and early diagnosis of subclinical patients are recommended (Baumgartner and Khol 2006).

By restricting animal movements and investigation of herds for pTB presence, MAP-free zones can be created, and the spread of paratuberculosis can be prevented (Baumgartner and Khol 2006, Yıldırım and Civelek 2013).

The intensity of subclinically infected animals is directly related to observed clinical pTB rate. The animals with subclinical pTB are critical for being prone to contaminate the infection to the healthy ones. Considering the zoonotic potential and resistance to pasteurization, to reveal subclinical infection presence in regions where intensive farming of dairy cows also is important (Yıldırım and Civelek 2013).

In the present study, ELISA, which is one of the cheapest and most practicable tests for herd control programs for MAP, has been used. In this research, it has been aimed to determine seroprevalence of subclinical paratuberculosis in Manisa region where intensive dairy cattle maintenance-farming is performed. Our research will also contribute to the development of effective control strategies against the disease across the region.

MATERIALS AND METHODS

Animal Materials

The animal material of the presented research was selected randomly from 40 different farms in Manisa region of Turkey (central district of Manisa; 10 farms, Akhisar; 5 farms, Kula; 5 farms, Alasehir; 2 farms, Turgutlu; 3 farms and Salihli; 15 farms). Research material consisted of 442 clinically healthy multiparous Holstein dairy cattle, 3.71±1.00 year-old,

with ongoing cyclic activities which did not have a periparturient disease (displacement abomasum, ketosis, retention and metritis) in previous lactation periods, and whose live weights were 445.42±37.28 kg and previous annual milk yields were 3576.60±373.82 kg. Distribution of the samples was as follows: Salihli, n=150; Akhisar, n=50; Kula, n=50; Turgutlu, n=25; Alaşehir, n=25 and the center of Manisa, n=142.

Blood samples were taken from each cattle colleting the material into plain biochemistry tubes. Extracted blood serums were stored at -20 °C until the analysis time.

Serologic Tests

In the diagnosis of the disease and in determining the seroprevalence, ELISA (Idexx Paratuberculosis Screening Ab Test, Part Number P07130-5 (5 plates) method was used. Applied ELISA test protocol is as follows:

Serum samples and all test kit reagents were brought to room temperature. Positive, negative control serums and serums to be tested were diluted with 1/20 sample dilution liquid (green). Being stirred, the samples were incubated at room temperature for one hour. After the incubation, control and test sera diluted to100 µl were transferred to microplates. Two wells were used for each positive and negative control serums. After being incubated at room temperature for 45 minutes, microplates were washed three times with washing solution. After being diluted with 1/100 conjugate dilution liquid (blue), Horseradish peroxidase (HRP) conjugate was transferred to washed microplate wells as 100 µl. The microplates with conjugate were incubated at room temperature for 30 minutes; and then, washing process was repeated. 100 µl of 3,3'- 5,5', -Tetramethylbenzidine (TMB) substrate were added to all wells and incubated in dark at room temperature for 10 minutes. At the end of the period, 100 µl of stop solution were added in the wells. Microplates were scaned in BIOTEK ELX800, USA at wavelength of 450 nm. Arithmetic mean (mean PC, mean NC) of optic density (OP) values of positive (PC) and negative (NC) control sera was calculated. S/P value for each of the serum samples was calculated with the formula; S/P= (Sample OD -mean NC) / (mean PC - mean NC). Calculated S/P ratios were evaluated as; S/P ≤0.60; Negative, S/P ≥0.70; Positive and S/P=0.6-0.7; Suspicious.

Statistical Analysis

In the calculation of age, live weight and previous lactation milk yield values, non-parametric 1 Sample (K-S) test (SPSS 16.0 for Windows) was utilized.

RESULTS

The obtained data have indicated the presence of pTB infection in all tested herds in Manisa region of Turkey. In this context, in the presented study, when the cattle that were sampled in each district were considered as a single herd, herd seroprevalence of MAP was found as 100%. While, within-herd seroprevalence was defined as 21.72%.

In the presented study, samples were collected randomly from 142 multiparous dairy cattle from five cowsheds in the central district of Manisa. 19 of the cattle were seropositive. Seroprevalence of subclinical paratuberculosis was identified as 13% in the central district of Manisa. In Turgutlu, 25 randomly selected multiparous dairy cattle from three cowsheds were sampled. Seven of the cattle were seropositive. Seroprevalence of subclinical paratuberculosis was identified as 28% in Turgutlu. In Alaşehir, 25 multiparous dairy cattle from three cowsheds were sampled. Five of the cattle were seropositive. Seroprevalence of subclinical paratuberculosis was 20% in Alaşehir. In Kula, 50 randomly selected multiparous dairy cattle from four cowsheds were sampled. 19 of the cattle were seropositive. Seroprevalence of subclinical paratuberculosis was identified as 38% in Kula. In Akhisar, 50 randomly selected multiparous dairy cattle from four cowsheds were sampled, and 13 of those were seropositive. Seroprevalence of subclinical paratuberculosis was identified as 26% in Akhisar. In Salihli, 150 randomly selected multiparous dairy cattle from 19 cowsheds were sampled. 33 of the cattle were seropositive. Seroprevalence of subclinical paratuberculosis was identified as 22%. When an overall assessment was made, in the presented study, seroprevalence of subclinical pTB in 442 clinically healthy dairy cattle tested in Manisa region was 21.72% (Table 1).

Table 1: Regional seroprevalence of MAP in Manisa province, Turkey.
Tablo 1: Manisa yöresi MAP seroprevalansı.

Manisa Province	n (total)	n (MAP +)	%
Central district	142	19	13.38
Turgutlu district	25	7	28
Akhisar district	50	13	26
Alasehir district	25	5	20
Salihli district	150	33	22
Kula district	50	19	38
Total	442	96	**21.72**

DISCUSSION

The presence of paratuberculosis in our country has long been known; yet, the number of scientific research conducted on the prevalence of pTB is quite limited (Fırat 1978, Cetinkaya et al. 1999, Civelek et al. 2009, Ozturk et al. 2010, Yıldırım and Civelek 2013, Makav and Gokce 2013). In the presented study, prevalence of subclinical pTB in Manisa, Turkey where dairy cattle farming is intensive has been investigated serologically using the method ELISA.

For the study, 442 clinically healthy, multiparous Holstein dairy cattle aging between 2-6 years with optimal milk yield and live weight were selected.

MAP is a strong intra-cellular pathogen. It survives in macrophage cells for a long time. Blood antibody level increases depending on the development of and the respond to infection. That makes the diagnosis of pTB difficult in the cattle under the age of two. For that reason, in the prevalence studies which blood serum is used as diagnosis material, animals under the age of two must not be selected as the material. The fact that seroprevalence of pTB has been low in the cattle under the age of two supports this case (Cetinkaya et al. 2000, Diequez et al. 2009). Besides, it has also been reported that the sensitivity and specificity of ELISA test are low in the cattle at the age of <2 years (Ozturk et al. 2010). In the present study, target population was selected as the dairy cattle at the age of >2 years.

pTB causes major economic losses particularly to dairy cattle enterprises. During the subclinical period, the animals with pTB are prone to contaminate healthy animals with the disease. The factor mainly spreads through fecal matter and milk (Moghadam et al. 2010). Therefore, determining seroprevalence of pTB in subclinic period is of vital importance for the management and health of the herd (Ozturk et al. 2010, Yıldırım and Civelek 2013). Besides, the MAP factors can also be transmitted to newborn calves in the neonatal period or immediately after birth. This period is when the calves are the most susceptible to infection. Contaminated nipple, colostrum or milk plays a role in the factor's being taken by the calf (Cetinkaya et al. 1997, Cetinkaya et al. 2000, Yıldırım and Civelek 2013).

MAP is also a potentially suspicious zoonotic factor. It is suspected to take part in the etiology of Crohn's disease in humans (Smith and Bradford 2001, Andrews 2004, Yıldırım and Civelek 2013). MAP that is resistant to heat and pasteurization can transmit to people through milk and dairy products. This disease's posing the risk of zoonosis makes the diagnosis of dairy cattle in subclinical period, in other words early diagnosis, important (Cetinkaya et al. 1997, Cetinkaya et al. 2000, Yıldırım and Civelek 2013).

In the study they conducted on the cattle in Central Anatolian Region, Atala and Akçay (2001) assessed the seroprevalence of pTB with micro and tube CFT test, and identified it as 2.3% and 2.7%, respectively. For this region seroprevalence of pTB was reported by ELISA method as 4.6%. Yıldırım and Civelek (2013) identified prevalence of subclinical pTB in fecal matter in Uşak region, Turkey as 17% by ZN staining, 9.5% by the technique of Outher PCR, and 20% by the technique of Nested PCR. Prevalence in milk samples were found as 15.5% by ZN staining, 5.5% by the technique of Outher PCR, and 17.5% by the technique of Nested PCR. Seroprevalence of pTB in milk samples in Elazığ region, Turkey was identified by Çetinkaya et al. (2000) as 5% with PCR method. The method of bacterial culture has demonstrated prevalence as 3.4%. Seroprevalence of pTB in serum samples in Kars region, Turkey has been identified as 3.5% by ELISA test (Makav and Gokce 2013).

Climate, nutrition and housing conditions are influential factors in prevalence of pTB (Cetinkaya et al. 1997, Cetinkaya et al. 2000). So, prevalence of pTB may vary in different regions.

In the studies carried out on pTB in Turkey, the prevalence in farms has not been taken into account much. Diagnosis of pTB even in one cattle in an enterprise raises the risk of infection greatly. Farm prevalence of pTB was identified as 58% in Burdur region, Turkey (Ozturk et al. 2010), and 41.6% in Kars region, Turkey (Makav and Gokce 2013). To generalize, the data demonstrates that paratuberculosis is seen in one out of every two enterprises in our country. Various studies carried out in European countries have reported that farm prevalence of pTB ranges between 0% and 75% (Diequez et al. 2009). It has been suggested that prevalence of pTB rises as the number of the animals in the farm rises (Nielsen, 2008, Ozturk et al. 2010).

Subclinical pTB has been present in all enterprises involved in the study. Prevalence of subclinical pTB is 21.72% in dairy cattle in Manisa region of Turkey. Moreover, if each one of the districts is considered as a single farm, because infection has been detected in all tested herds, seroprevalence can be identified as 100% depending on the research data.

As a result, the fact that seroprevalence of MAP infection in dairy cattle in Manisa region of Turkey has been identified as 21.72% and all farms sampled have been seropositive is of vital importance for the region where intensive dairy cattle farming is performed.

Many of the subclinically infected animals do not show clinical symptoms of pTB all their lives. The infection manifests itself clinically only in 5-10% of the subclinically infected animals in a herd (Baumgartner and Khol 2006). However, these animals play a role in the spread and contamination

of the factor to other animals. Diagnosis of the subclinical animals is critical because of their being prone to transmit the infection to healthy animals. Futhermore, considering the zoonotic potential of the infection and resistance of the factor against pasteurization (Chiodini and Hermon-Taylor 1993, Grant et al. 1996), subclinical pTB in the cattle is also critical for the public health.

Higher values of subclinical pTB identified in this study compared to other studies conducted in Turkey suggests that Manisa region carries a substantial risk in terms of infection's contamination potential. This study carried out in Manisa region will also contribute to predict economic losses caused by the disease and identify prevalence of the infection in Turkey.

CONCLUSIONS

Consequently, the obtained data suggest that paratuberculosis posing a threat to economy in Manisa region must be taken into consideration. The results of the study emphasize the necessity of a further nation-wide research in order to reveal the presence of subclinical pTB in the country's dairy cattle population and to define its real damage to the national economy.

REFERENCES

Allaker RP, Kapas S. Adrenomedullin expression by gastric epithelial cells in response the infection. Clin Diagn Lab Immunol 2003; 10: 546-551.

Andrews AH. Johne's disease (Paratuberculosis). In: Bovine Medicine, Ed; Eddy RG, Blackwell Scientific Publication, London, England, 2004; pp. 857-858.

Atala N, Akcay E. Türkiye genelinde sığır paratuberkulozu prevalansının ELIZA ile araştırılması. Etlik Vet Mikrobiyol Derg 2001; 12: 39-48.

Baumgartner W, Khol JL. Paratuberculosis (Johne disease) in ruminants ongoing story. Slow Vet Res 2006; 43: 5-10.

Cetinkaya B, Erdogan HM, Morgan KL. Risk factors for Bovine Paratuberculosis. The multiple analysis of risk ractors for Bovine Paratuberculosis. Turk J Vet Anim Sci 1997; 21: 303-306.

Cetinkaya B, Ergan K, Harbour DA. An abatoir-based study of the prevalance of subclinical Johnes diesease in adult cattle in south west England. Epidemiol Infect 1999; 116: 373-379.

Cetinkaya B, Muz A, Ertas HB. Süt ineklerinde Paratüberküloz prevalansının Polimeraz Zincir Reaksiyonu (PZR) ile saptanması. Turk J Vet Anim Sci 2000; 24: 371-379.

Chiodini RJ, Hermon-Taylor J. The thermal resistance of Myobacterium paratuberculosis in raw milk under contidions simulating pasteriuzation. J Vet Diagn Invest 1993; 5: 629-631.

Civelek T, Çelik HA, Özenc E, Avcı G, Kav K, Cıncı CC. Effects of PCR-confirmed subclinical paratuberculosis on retinol and β-carotene levels in dairy cattle. Arch Med Vet 2009; 41: 281-284.

Collins MT, Socket DC, Goodger WJ. Herd prevalence and geographic distribution of, and risk factors for bovine paratuberculosis in Wisconsin. J Am Vet Med Assoc 1994; 204: 636-641.

Collins MT, Wells SJ, Petrinil KR. Evaluation of five antibody detection tests for diagnosis of bovine paratuberculosis. Clin Diagn Lab Immunol 2005; 12: 685-692.

Diequez FJ, Gonzales AM, Menendez S. Evaluation of four commercial serum ELISAs for detection of *Mycobacterium avium subsp. paratuberculosis* infection in dairy cows. Vet J 2009; 180: 231-235.

Fırat G. Sığırlarda paratüberkülozun serolojik olarak teşhisi üzerine araştırmalar. Pendik Vet Bak Ser Enst Dergi 1978; 1: 18-23.

Grant IR, Ball HJ, Neil SD. Inactivation of Myobacterium paratuberculosis in cows milk at pasteurazition temperatures. Appl Environ Microbiol 1996; 62: 631-636.

Hacker U, Huttner K, Konoe M. Untersuchungen zur serologischen Prävalenz und zu Risikofaktoren der Paratuberkulose in Milchviehbetrieben in Mecklenburg Vorpommern. Berl Münch Tierärztl Wochenschr 2004; 117: 140-144.

Jakobsen MB, Alban L, Nielsen SS. A cross-sectional study of paratuberculosis in 1155 Danisch dairy cows. Prev Vet Med 2000; 46: 15-27.

Jubb TF, Sergeant ES, Callinan AP. Estimate of the sensitivity of an ELIZA used to detect johne's disease in Victorian dairy cattle herds. Aust Vet J 2004; 82: 569-573.

Kalis CHJ, Hesselink JW, Barkema HW. Comparison of culture of individual and strastegically pooled bovine faecal samples for *Mycobacterium avium subsp. parastuberculosis*. In: Proceedings of the 6th International Colloquium on Paratuberculosis. Madison, Wisconsin, USA, 1999; pp. 344-348.

Makav M, Gokce E. Kars yöresi sığırlarında subklinik paratüberkülozun seroprevalansı. Kafkas Univ Vet Fak Derg 2013; 19: 913-916.

Mecitoglu Z, Demir G. Sığırlarda paratüberkülozun tanısına ilişkin problemler. J Fac Vet Med 2012; 1: 19-23.

Moghadam MT, Sarv S, Moosakhani F. Detection of Mycobacterium avium Subspecies paratuberculosis in Milk and facel Samples in Dairy Cattle by PCR and Nested-PCR. J Anim Vet Adv 2010; 9: 3055-3061.

Muskends J, Mars MH, Elbers AR. The results of using faecal culture as confirmation test of paratubercu-losis-seropositive dairy cattle. J Vet Med B Infect Dis Vet Public Health 2003; 50: 231-241.

Nakase H, Nishio A, Tamaki H. Specific antibodies against recominant protein of insertion element 900 of Mycobacterium avium subspecies paratuberculosis in Japanese patients with Crohn´s disease. Inflamm Bowel Dis 2006; 12: 62-69.

Nielsen SS. Transitions in diagnostic tests used for detection of Mycobacterium avium subsp. paratuberculosis. Vet Microbiol 2008; 132: 274-282.

Ozturk D, Pehlivanoglu F, Tok AA. Seroprevalence of paratuberculosis in the Burdur province (Turkey), in dairy cattle using the enzyme linked immunosorbent assay (ELIZA). Israel J Vet Med 2010; 65: 53-57.

Smith S, Bradford P. Johnes Disease. In: Large Animal Internal Medicine. Ed; Smith S, Mosby, St.Louis, Missouri, USA:, 2001; pp.779-783.

Stricklands J, Scott HM, Mcjordan ER. Effects of seasonal climatic conditions on the diagnosis of Mycobacterium avium subspecies paratuberculosis in dairy cattle. J Dairy Sci 2005; 88: 2432-2440.

Yıldırım D, Civelek T. Prevalence of subclinical paratuberculosis in dairy cattle in Uşak Region. Kafkas Univ Vet Fak Derg 2013; 19(1): 121-126.