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F. BUYUK, K. BOZUKLUHAN, A. GULMEZ SAGLAM, G. GOKCE, O. CELEBI, E. CELIK, S. KIZILTEPE, M. R. COSKUN, S. OTLU, M. SAHIN

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The prevalence estimates of *Mycobacterium bovis* infection in cattle with ELISA

Buyuk F.[1*], Bozukluhan K.[2], Gulmez Saglam A.[1], Gokce G.[3], Celebi O.[1], Celik E.[1], Kızıltepe S.[3], Coskun M.R.[1], Otlu S.[3], Sahin M.[1]

[1] *Department of Microbiology, Faculty of Veterinary Medicine, Kafkas University, TR-36100, Kars, Turkey.*

[2] *Kars Vocational School, Kafkas University, TR-36100, Kars, Turkey.*

[3] *Department of Internal Medicine, Faculty of Veterinary Medicine, Kafkas University, TR-36100, Kars, Turkey.*

ABSTRACT. Tuberculosis is an infectious bacterial disease of *Bovidae* and the causative agent is *Mycobacterium bovis*. It is responsible for remarkable economic losses among cattle herds with widely dispersion. Prompt and consistent diagnosis of tuberculosis especially in countries where the disease is endemic as in Turkey is of great importance to detect and identify infectious cases for strengthening control measures. In the present study, it was aimed to detect true animal and herd prevalence (within-herd, and between-herd) of antibodies against *M. bovis* in cattle herds. A serologic survey for antibody detection against the *M. bovis* was conducted by using an ELISA kit. Thirty three cattle herds were randomly selected from different farms and totally 460 cattle over five years of age were sampled. The true animal, within-herd, and between-herd prevalences found were 5.9% (95% CI = 3.0 to 8.8), 11.1% (95 CI = 6.5 to 15.8) and 73.4% (95 CI = 51.2 to 95.6), respectively. Results will provide useful information about the status of *M. bovis* infection and will contribute to the disease control practices.

Keywords: tuberculosis, cattle, prevalence estimates, ELISA

Corresponding Author:
Fatih Buyuk, Department of Microbiology, Faculty of Veterinary Medicine,
Kafkas University, TR-36100, Kars, Turkey.
E-mail: fatihbyk08@hotmail.com

INTRODUCTION

Bovine tuberculosis is a chronic infectious disease of cattle with a universal dispersion. *Mycobacterium bovis* is the causative agent of the disease in this species. In addition to large economic losses in livestock management, it poses a major public health concern with defined zoonotic aspect (Souza et al., 2012).

Though the disease is nearly eliminated in many countries including Australia, Sweden, Slovakia, Canada, etc., it is widespread in Africa, Asia and some Middle East countries (Schiller et al., 2010). According to World Organization for Animal Health (OIE) data, Turkey is one of the countries where tuberculosis exists. The infection rate of the year 2011 was reported as 22.8% in cattle population in Turkey (OIE, 2011).

The disease can be transmitted by the inhalation of aerosols, by ingestion, or through cracks in the skin (Phillips et al., 2003). Large numbers of organisms may be shed in the late stages of infection. The course of the disease is usually chronic and cattle can remain asymptomatic and anergic for a long period of time. Few animals become symptomatic and it is mostly diagnosed by routine tests or found infective at the slaughtering (Schiller et al., 2010). The best mode to control of bovine tuberculosis is accurate diagnosis and disposal of the infected animals with 'test and slaughtering' programmes (OIE, 2004). Herewith, the influential ante-mortem surveillance of bovine tuberculosis must primarily rely on the diagnosis of the infected cattle at an early stage using of sensitive immunodiagnostic methods (Adams, 2001).

Testing of cattle using the purified protein derivative (PPD)-tuberculin is the most referenced method implemented in disease control programmes. The tuberculin skin test is used widely for this purpose. Additionally, gamma interferon test (γ- IFN) is the other principal mediator of cellular immunity. However, all these cell-mediated immune (CMI) detectors are not efficient in detecting the disease at its different stages particularly at its advanced stage where the CMI response decreases and humoral response, which produces antibodies, predominates (Wadhwa et al., 2012). Withal, some defined insensitivities such as immunosuppression,

desensitization and false-positive reaction due to exposure of animals to *Mycobacterium avium* or *Mycobacterium avium* subspecies *paratuberculosis (MAP)* and some application problems such as difficulties in intradermal challenge, evaluation of skin thickness and two times handled of animals for test repeat have arisen during the use of CMI based assays (Monaghan et al., 1994; Ozturk et al., 2010; Wadhwa et al., 2012). Hence the use of a serological test like ELISA has been enounced as an alternative testing method for tuberculosis in cattle (Sayin and Erganis, 2013).

In this study, the prevalence estimates of *M. bovis* infection among cattle herds were conducted in Kars City, Turkey. For this purpose, a commercially available ELISA kit was used.

MATERIALS AND METHODS

Study design

This study was approved by the local ethical committee of animal experiments at Kafkas University (Protocol no. KAU-HADYEK/2012-23). Randomly sampling method was used for animal selection. In this context, >5 years old cattle were selected from farms, where extensive rearing system (stock farming mainly based on pasture and meadows) is implemented. The minimum sampling size (number of cattle) was estimated as 383 using a confidence level of 95% and confidence interval (CI) of 5% and considering the total cattle number of Kars Region as approximately 575,000 (data were obtained from the Kars Province of Food, Agriculture and Animal Husbandry Department).

Animal Sampling

Animal material of the study is consisted of 460 adult (over 5 years) cross-breed female cattle provided from 33 herds in Kars and its counties. In brief, 110 blood samples from 10 herds of Kars center and 350 samples from 23 herds of all counties were used (Table 1). Herds were non-vaccinated against *M. bovis* or *MAP* and were not submitted to tuberculin testing earlier. Blood samples were collected from jugular vein of animals into 5 ml vacuum tubes without anticoagulant (BD, Turkey) and forwarded to the Microbiology laboratories of Kafkas University

and serum samples were separated after 10 minutes centrifugation at 3000 rpm and kept at -20 °C till analysis.

ELISA

A commercially available ELISA kit (Idexx, USA) recommended by OIE with a confirmation number of 20120107 (OIE, 2012) was used to detect antibodies against *M. bovis*. To prevent waste of the ELISA, all kit wells were utilized with testing 460 samples. Briefly, serum samples and kit controls were 1:50 diluted with the dilution buffer, 100 µl diluents were transferred to the ELISA plate and incubated at room temperature (22-26 °C) for one hour. Plates were washed with wash buffer, loaded with 100 µl monoclonal anti-bovine IgG conjugate and incubated at room temperature for 30 minutes. Plates were washed once again and loaded with 100 µl TMB substrate and incubated for 15 minutes and the reaction was terminated by addition of 50 µl stop solution. Plates were then read at 450 nm wavelength and the results were recorded. Results were calculated as sample-to-positive control ratio (S/P) derived by subtracting the mean negative-control OD value from each sample and dividing this by the corrected positive-control OD value (this was the value of mean positive control OD minus mean negative control OD). The samples ODs were then compared with the kit positive control OD to derive S/P ratios. Sample with an S/P ratio of ≥ 0.30 was considered positive for *M. bovis* antibodies.

Statistical analysis

The data were loaded into Microsoft Excel 2010 and transferred to SPSS® Version 20 for statistical analysis. Statistical differences of ELISA results were measured by the Chi square test. *P*-values smaller than 0.05 were accepted statistically significant. The sensitivity and specificity of *M. bovis* antibody test was determined at the cut-off values established by the manufacturer (Idexx, USA).

The case definition and subsequent serial calculations of the apparent individual and mass prevalences (within-herd and between-herd) were carried out by the method reported by Buyuk et al. (2014). True animal, within-herd, and between-herd

prevalences were calculated using the Rogan-Gladen estimator (Rogan and Gladen, 1978). The ELISA kit sensitivity (77.8%) and specificity (98.2%) as reported by the manufacturer was considered when true prevalence was estimated.

RESULTS

Totally, 460 cattle from 33 herds namely 110 cattle from 10 center and 350 cattle from 23 county farms of Kars City were analyzed. Out of 460 cattle tested 29 were found positive in terms of *M. bovis* antibodies. The positive animal distributions of center and county farms were 5 and 24, respectively. The number of cattle detected with antibodies against *M. bovis* between the center and county farms was statistically insignificant ($\chi2 = 0.675$, P = 0.411). Among 33 herds tested, 19 were found having at least one or more *M. bovis* positive cattle, while 14 herds were tuberculosis-free. The number of animals in seropositive herds is 283 whereas seronegative herds had 177 animals. These numerical values were used to calculate animal, within-herd and between-herd prevalence (Table 1).

As a result, the apparent prevalences of animal, within-herd, and between-herd were found 6.3% (95% CI = 4.4 to 8.9%), 10.2% (95 CI = 7.2 to 14.3%) and 57.6% (95% CI = 40.8 to 72.8%), respectively. The true prevalences of animal, within-herd, and between-herd were calculated as 5.9% (95% CI = 3.0 to 8.8), 11.1% (95 CI = 6.5 to 15.8) and 73.4% (95 CI = 51.2 to 95.6), respectively (Table 2).

DISCUSSION

Bovine tuberculosis still continues to be a problem with global appearance in spite of intensive eradication efforts over decades (Schiller et al., 2010). In Turkey, the disease prevalence is reported at the rate of 22.8% countrywide (OIE, 2011). There are not sufficient and comprehensive studies in Kars Region. In a pathological study, bovine tuberculosis is found at the rate of 0.9% in slaughtered cattle (Beytut, 2001). The other report about Kars was conducted by Unver et al. (2007) and 6.7% positivity was reported in lung and mediastinal lymph node samples of slaughtered cattle by PCR. In this study, the true animal prevalence with a percentage of 5.9%

Table 1: The sample layouts and existing prevalences of whole Kars Region

Locality	Tested		Seropositive		Apparent prevalence		True prevalence	
	Farm	Animal	Farm	Animal	Estimate, %	95% CI	Estimate, %	95% CI
Center	10	110	5	5	4.5	2.0-10.2	3.6	<0-8.7
Akyaka	3	50	3	4	8	3.2-18.8	8.2	<0-18.1
Arpaçay	4	50	4	7	14	7.0-26.2	16.1	3.4-28.7
Digor	3	50	1	3	6	2.1-16.2	5.5	<0-14.2
Kağızman	3	50	3	5	10	4.3-21.4	10.8	<0-21.7
Selim	4	50	2	4	8	3.2-18.8	8.2	<0-18.1
Sarıkamış	2	50	-	-	-	-	-	-
Susuz	4	50	1	1	2	0.4-10.5	0.3	<0-5.4
Total	33	460	19	29	6.3	4.4-8.9	5.9	3.0-8.8

shows a great harmony with both Kars Region and countrywide results (Unver et al., 2007; OIE, 2011). It is allowable that the disease moves about less than 10% in given region among live animals. With a moderate infection rate it poses a risk to spread within herds which have already had a prevalence rate as 11.1%. Due to the contagious nature of the bovine tuberculosis, the within and between-herd transmission is always possible by continuous new infection among adult animals, high seroprevalence with eventual environmentally contamination and free and immense interzonal movement of animals. Thus, it makes the results significant indicating that *M. bovis* infection is widespread in cattle population in the Kars District.

The immunity is dominated by cell-mediated response in early stage of infection in cattle exposed to *M. bovis*. Mainly cell-mediated immune response detectors (skin and c-IFN test) are used to identify the positive cattle (Alito et al., 2003). The immunity is subsequently shifting towards an antibody-based response, in parallel with the progression of infection (Welsh et al., 2005). The cell-mediated methods become less sensitive in the advanced phase of disease, when it can be diagnosed serologically (mainly enzyme immunoassay= ELISA). On the other hand, the proportion of 'anergic' cattle, which are likely to be highly infective and non-responsive to the CMI-based tests, can't be ignored. Thus, it makes favorable to use tests which are able to detect antibody response. The ELISA technique that has been applied for the diagnosis of bovine tuberculosis and claimed extremely advantageous to identify infected cows, enables their separation from the herd and assists disease eradication (Lilenbaum L and Fonseca, 2006; Wadhwa et al., 2014). This study was conducted in adult cattle, all were over the age of 5 years, attempting to detect the animals in advanced phase of disease through the specific antibody response as the final outcome of infection. Therewith the animal prevalence of bovine tuberculosis was found as 5.9% around of Kars Region. Though the positive animals' rate is low, they still pose risk for the remaining population.

From a different viewpoint, the interpretation of a serological test is difficult because of some false positive or negative results that can arise when using estimate of prevalence of a disease. Thus, a necessity is arisen to distinguish the true prevalence (the proportion of a population that is actually infected) and apparent prevalence (the proportion of the population that tests positive for the disease) (Speybroeck et al., 2013). With a test sensitivity (se) as 77.8% and specificity (sp) as 98.2% the true prevalence was calculated versus apparent prevalence values in this study. By using the estimates obtained by the Rogan-Gladen estimator (Rogan and Gladen, 1978), it was possible to estimate the true prevalence

Table 2: Varied prevalence estimates detected in this study

Prevalence type	Tested animal	Seropositive animal	Apparent prevalence		True prevalence	
			Estimate, %	95% CI	Estimate, %	95% CI
Animal	460	29	6.3	4.4-8.9	5.9	3.0-8.8
Within-herd	283	29	10.2	7.2-14.3	11.1	6.5-15.8
Between-herd	33	19	57.6	40.8-72.8	73.4	51.2-95.6

of bovine tuberculosis in the population without sampling all animals.

CONCLUDING REMARKS

The prompt diagnosis of tuberculosis especially in countries where the disease is endemic as in Turkey is of great importance to detect and identify infectious cases. Due to the inadequacies in CMI based diagnosis in advanced phase of tuberculosis the prevalence studies as presented herein with using an ELISA technique will provide useful information about the current status of *M. bovis* infection and will contribute to the disease control practices.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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