SEROPREVALENCE OF PARATUBERCULOSIS IN THE BURDUR PROVINCE (TURKEY), IN DAIRY CATTLE USING THE ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

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ABSTRACT

The objective of this study was to determine the seroprevalence of paratuberculosis in dairy cattle in Burdur province located in southwest of Turkey. Blood samples were collected and tested by the enzyme linked immunosorbent assay (ELISA) from 465 dairy cattle older than 2 years randomly selected from 24 dairy cattle herds. Seroprevalence of paratuberculosis was found in 6.2% at the individual cow level and 58.3% at herd level. Seropositivity was detected in a total 14 of 24 herds. The prevalence of paratuberculosis by age ranged from 3.6% to 19.73% where the highest prevalence rate was found in 3 years olds. In conclusion, we determined that the seroprevalence of paratuberculosis in Burdur province was higher than those in other parts of Turkey. In addition, we found that when the size of the herd increased, the possibility of infection with paratuberculosis was greater.

Key words: Paratuberculosis, dairy cattle, ELISA.

INTRODUCTION

Bovine paratuberculosis is a chronic disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (1, 2). Paratuberculosis causes large economic losses due to reduction in milk production, increased veterinary costs, diarrhea and weakness in infected animals. Paratuberculosis is introduced to herds by means of infected cattle which may or may not present with clinical signs. Younger cattle of less than 30 days old are susceptible to infection and do not show any clinical signs until they are at least 3-5 years old (3, 4, 5).

The diagnosis of subclinical paratuberculosis is generally difficult due to a long incubation perod, non-specific clinical signs, late immune response, and the intermittent emission of the agent (3,4,6). Bacteriological culture methods have a high specificity (~100 %) in faecal samples and is commonly used as reference test (3). The infected animals which are serologically and clinically negative can be detected by fecal culture (3). Bacteriological culture requires a long incubation period, and has a considerably high risk for contaminations with high costs and therefore not generally used (4,5,7,8). Serological tests such as agar gel immunodiffusion (AGID), complement fixation (CFT), enzyme linked immunosorbent assay (ELISA) are used for diagnosis of paratuberculosis (3,7,9,10,11,12,13). ELISA is most commonly used for the diagnosis of paratuberculosis as a serologic assay as it is cheap and easy to perform (3,14,15,16) and more sensitive than the AGID and CFT for diagnosis of clinically affected animals (3).

In this study we set out to determine the prevalence of paratuberculosis by the ELISA technique in dairy cows in Burdur province of Turkey. We also investigated the relationship between herd size and paratuberculosis.

MATERIALS AND METHODS

Animals and sample collection

This study was conducted on 24 Holstein dairy cattle herds in the Burdur province, located in southwest of Turkey. The total of area of the region is 7,135 km^2 and is a crossing area between the Aegean, Middle Anatolia and Mediterranean parts of Turkey. The maximum altitude is 1000 m above sea level. The herds with cattle two years and older animals were randomly selected without consideration of herd size. Sera samples were collected from four hundred and sixty five animals.

Since there were no studies in Burdur province on the prevalence of paratuberculosis in dairy cattle herds, the sample size of animals was determined using an expected seroprevalence of 50%, a confidence level of 95% and error of

5%. The number of animals required by the method was 384 (17), however, 465 animals older than 2 years old belonging to 24 herds were sampled to increase the precision. The population of herds is summarized in Table1.

ELISA

The serum samples were tested by a commercial ELISA kit (ELISA Paratuberculosis Screening Test®, Institut Pourquier, France) which was used to assay antibodies to *M. avium* subsp. *paratuberculosis* according to manufacturer instructions. According to manufacturer instructions, the samples were diluted and incubated in a dilutions of buffer containing a *Mycobacterium phlei* extract in order to eliminate the cross reactions. After incubation, one negative, two positive controls and the samples were placed in the wells of the microplates. Positive and negative controls were used in each plate. The absorbance values (OD) were measured using a 450 nm filter within 5 minutes of terminating the reaction. The mean OD values of positive controls and the OD value of negative control were checked for validation of the test which was 0.350 for the positive control and the mean OD_{450} value of the positive controls/OD_{450} value of the negative control was ≥3. For each sample, the ratio S/P (S/P=100x(OD_{450} of the sample-OD_{450} of the negative control)/(mean OD_{450} of the positive control–OD_{450} of the negative control)) was calculated. According to the test protocol, S/P ratios equal or lower than 60% were considered negative for paratuberculosis. For S/P ratios between 60% and 70% the result was considered uncertain. The samples with an S/P ratio of greater than or equal to 70% S/P were regarded as positive for paratuberculosis.

RESULTS

The prevalence for paratuberculosis among cattle tested in the Burdur province was found to be 6.2% (29/465) by the ELISA test. Seropositivity was detected in total 14 of 24 herds. The total number of animals in the positive herds was 332 (Table 2). Nine herds had only one seropositive cow and five herds had two or more seropositive animals (Table 2). When analyzed by age the highest prevalence value of 19.73% (15/76) was determined in 3 years old, the lowest prevalence value 3.6% (3/84) was detected in 2 years old. The seroprevalence of animals by age is presented in Table 2. While the prevalence of paratuberculosis at the herd level was high (100%) in the herds of greater or equal to 81 animals it was quite low (20%) in the herds with 20 animals or less. Herd level prevalence values were presented in Table 3.

DISCUSSION

Paratuberculosis causes important economic losses in ruminants (2,18). Although the presence of paratuberculosis has been known for a long time in Turkey, there are a few studies related to prevalence of paratuberculosis (6,9,10,12). In Middle Anatolia region, Firat (1978) and Vural and Atala (1988) reported the prevalence of paratuberculosis in cattle by as 2.7% and 4.3%, respectively using the complement fixation test (9, 12). In Elazig province, Çetinkaya et al. (2000) found the prevalence of infection to be 5.0% by PCR and 3.4% by bacteriological culture (7). Ikiz et al. (2005) reported that paratuberculosis could not be detected by PCR from fecal samples of randomly selected cattle in the Thrace region (11). In the present study, we showed that the prevalence of paratuberculosis was 6.2% by ELISA in the Burdur province and in fact higher than those in other parts of Turkey. In Slovenia, Ocepek et al. (2002) reported cattle level prevalence as 3.4% in 1991 (3). In Alberta, Sorensen et al (2003) reported that prevalence of paratuberculosis was 8.1% in dairy cattle herds (10). McKenna et al. (2004) stated that prevalence of paratuberculosis was 15.1% and 21.7% in dairy cows in Atlantic Canada and Maine, respectively (1). Nielsen and Toft (2009) reported that cattle level prevalence of paratuberculosis changes from 3% to 20% in several European countries (2). We consider that the differences in prevalence values may have arisen from the geographic variations, location of farms and the use of different detection methods. This study supports research which regards that prevalence of paratuberculosis can be affected by the factors such as climate, nutrition, age, region and housing conditions and diagnostic methods (1,2,3,7,10).

In the present study, prevalence of paratuberculosis was found to be 8.7% in paratuberculosis positive herds. However, within herd prevalence of paratuberculosis varied between 3.6% and 19.7% in the positive herds. The highest prevalence value was determined in 3 years old animals. Our findings are supported by those of several researchers (1,4,19) who reported that the prevalence of paratuberculosis can increase with age of the animals. The prevalence of infection was the lowest in 2 years old animals and this can be reason for the presence of negative herds as the majority of the animals in these herds were two years or younger.

It has been stated that the sensitivity and specificity of ELISA is low in cattle younger than 2 years old, due to their late immune response. Several researchers (1, 22, 23) have reported that increase in the antibody response depends directly on the disease progress. In the present study, prevalence differences can be related to herd size and differences in age of animals in herds. Furthermore, several researchers (19, 20, 22) have reported that the prevalence of paratuberculosis increases with the size of the herds. In Slovenia, Ocepek et al. (2002) reported the herd level prevalence as 4.0% in 1999, and 11.6% in 2001. Woodbine et al. (2009) reported that herd level prevalence was approximately 75 % in southwest England. In this study, herd prevalence was 58.3%. Our findings concord with those who reported that herd prevalence was higher than >50% (2, 19). Woodbine et al. (2009) stated that prevalence of paratuberculosis was lower in cattle herds with ≤100 animals. In the present study, it was determined that cattle herds larger than 80 animals were seropositive. In contrast, only 20% of the cattle herds with ≤20 animals had at least one positive animal. This finding supports the studies (19, 20, 22) which

state that the possibility for being positive for paratuberculosis increases with herd size. The reason for this may be related to the introduction of new animals into the herd from different origins. In Burdur, the majority of the dairy cattle managements are small family farms. The calves after births are kept with dams and fed with cow milk. This may result in vertical transmission of the infection.

In recent years, ELISA has become the most commonly used serological assay for the diagnosis of paratuberculosis, because it is cheap, easy to perform, and has high specificity (5,6,11,13,14,15). Several researchers (2, 12, 21, 22, 23) have reported that the sensitivity and specificity of ELISA varies between 47%-51% and 95%-99%, respectively. It has been reported that ELISA should be used for the detection of paratuberculosis in geographical areas known to be free of paratuberculosis, due to its high specificity (13). In the present study, cattle with no clinical signs were found to be positive by the ELISA. According to the manufacturer, the kit has a sensitivity >50% and specificity >99.0% in milk and serum sample. Therefore, we thought that ELISA with high specificity can be used for diagnosis of paratuberculosis.

In conclusion, we determined that prevalence of paratuberculosis in the Burdur province is higher than those in other parts of Turkey, and as the size of the herds increases, the possibility for being infected by the paratuberculosis agent is enhansed.

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Table 1: Size and distribution of age of the cattle in the herds

Herds	Size of herds (n)	Age (years) of animals tested						
		2	3	4	5	6	≥7	Total (n)
1.	35	2	4	2	1	1	5	15
2.	77	-	3	5	2	4	12	26
3.	50	-	4	4	-	1	-	9
4.	47	1	3	4	2	4	1	15
5.	66	8	3	3	1	2	3	20
6.	54	18	2	3	2	-	1	26
7.	50	5	3	-	1	-	1	10
8.	29	5	1	2	4	1	1	14
9.	39	16	-	1	-	1	-	18
10.	10	1	-	4	1	-	-	6
11.	20	4	6	2	-	-	-	12
12.	25	2	2	3	-	2	3	12
13.	56	6	4	5	5	1	1	22
14.	31	5	4	-	2	-	2	13
15.	41	6	2	3	1	-	1	13
16.	13	-	11	-	2	-	-	13
17.	52	12	1	2	2	1	2	20
18.	97	15	8	5	16	7	4	55
19.	83	13	6	6	9	4	1	39
20.	28	4	5	3	-	-	2	14
21.	19	3	1	1	1	-	-	6
22.	17	8	3	-	-	-	-	11
23.	93	1	27	19	-	2	1	50
24.	64	6	3	11	6	-	-	26
Total	**1096**	**141**	**106**	**88**	**58**	**31**	**41**	**465**

Table 2: Distribution of seroprevalence in paratuberculosis positive herds by age

n=number of the animal tested, p=prevalence.

No. herds	Age (years) of animal tested												Total	
	2 year		3 year		4 year		5 year		6 year		≥7 year			
	Positive	n	Positive	n	Positive	n	Positive	n	Positive	n	Positive	n	Positive	p
1	1	2	0	4	0	2	1	1	0	1	0	5	2/15	13.3%
2	0	0	1	3	1	5	0	2	0	4	1	12	3/26	11.5%
3	0	0	1	4	0	0	0	0	0	1	0	0	1/9	11.1%
4	0	1	0	3	1	4	0	2	0	4	0	1	1/15	6.7%
6	0	18	0	2	0	3	1	2	0	0	0	1	1/26	3.8%
7	0	5	1	3	0	0	0	1	0	0	0	1	1/10	10.0%
8	1	5	0	1	0	2	0	4	0	1	0	1	1/14	7.1%
13	0	6	0	4	0	5	1	5	0	1	0	1	1/22	4.5%
18	0	15	3	8	0	5	0	16	1	7	1	4	5/55	9.1%
19	0	13	1	6	0	6	0	9	0	4	0	1	1/39	2.6%
20	1	4	2	5	0	2	1	1	0	0	0	2	4/14	28.6%
22	0	8	1	3	0	0	0	0	0	0	0	0	1/11	9.1%
23	0	1	5	27	1	19	0	0	0	2	0	1	6/50	12.0%
24	0	6	0	3	1	11	0	6	0	0	0	0	1/26	3.8%
Total	**3(3.6%)**	**84**	**15(19.73%)**	**76**	**4(6.06%)**	**66**	**4(8.16%)**	**49**	**1(4%)**	**25**	**2(6.66%)**	**30**	**29/332**	**8.7%**

Table 3: Classification of cattle herds based on size and herd level seroprevalence.

Size of herds (n)	Number of herds tested (n)	Seroprevalence (n) (%)
≤20	5	1 (20)
21-40	6	3 (50)
41-60	7	5 (71.4)
61-80	3	2 (66.7)
≥81	3	3 (100)
Total	**24**	**14 (58.3)**