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Short Paper

Prevalence of ELISA-detected specific antibodies against *Besnoitia besnoiti* in cattle of the Eastern and Southeastern Anatolian regions, Turkey

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Summary

Background: Besnoitiosis caused by *Besnoitia besnoiti* is regarded as a re-emerging disease in cattle because of the increased number of cases and geographical distribution in many European countries. **Aims:** The present study was conducted to determine the presence of *B. besnoiti* in cattle in the Eastern and Southeastern Anatolia of Turkey. **Methods:** Blood samples were collected from 450 cattle in the provinces of Mus, Van, Siirt, and Diyarbakir. PrioCHECK®Besnoitia Ab 2.0 enzyme-linked immunosorbent assay (ELISA) kit was used to detect specific anti-*B. besnoiti* antibodies in the serum samples. **Results:** Twelve (2.7%) of the 450 asymptomatic cattle were seropositive against *B. besnoiti*. In cattle, the prevalence rates were 0%, 3.7%, 3.4%, and 1.1% in Mus, Siirt, Diyarbakir, and Van provinces (P>0.05), respectively. This study is the first to investigate the presence of *B. besnoiti* in cattle raised in the Eastern and Southeastern Anatolia of Turkey. **Conclusion:** Although the ELISA test revealed some positive cases, concrete evidence for the establishment of clinical *B. besnoiti* infection could not be verified. More comprehensive analysis would be necessary to determine the significance of the present observations.

Key words: *Besnoitia besnoiti*, Cattle, ELISA, Seroprevalance

Introduction

Besnoitiosis is a disease characterised by symptoms such as sterility, hyperkeratosis, alopecia, and cysts in the connective tissues (Jacquiet *et al*., 2010; Alvarez-Garcia *et al*., 2013; Cortes *et al*., 2014). *Besnoitia besnoiti* pathogenicity leads to significant impact on the economy of the livestock industry (Cortes *et al*., 2005). This parasite, like *Neospora caninum* and *Toxoplasma gondii*, has an indirect life cycle in cattle (Hosseininejad and Hosseini, 2011; Papadopoulos *et al*., 2014). However, as neither dogs nor cats could be confirmed as definitive hosts of *B. besnoiti*, the epidemiology of this disease has not been fully understood (Basso *et al*., 2013).

Bovine besnoitiosis is widespread in Africa, the Middle East and Asia and has lately lately been admitted as a re-emergent cattle disease in Europe due to the increased number of cases (European Food and Safety Authority (EFSA), 2010; Alvarez-Garcia *et al*., 2013). Besnoitiosis is endemic in Spain and France (Alzieu *et al*., 2007; Fernandez-Garcia *et al*., 2010), while isolated outbreaks have been reported in Germany, Switzerland and Italy (Mehlhorn *et al*., 2009; Alvarez-Garcia *et al*., 2013; Rinaldi *et al*., 2013). Caprine besnoitiosis was also reported in Iran, which is a neighboring country to the east of Turkey (Oryan and Sadeghi, 1997; Namazi *et al*., 2011). However, in Turkey, only one study has been carried out in Kirikkale (Ocal *et al*., 2016). The EFSA report noticed that some aspects of the epidemiology of bovine besnoitiosis remain unclear, including the prevalence, transmission paths, and risk factors (EFSA, 2010).

Several diagnostic tests such as cytology, histopathology, serology and PCR testing have been used to diagnose of besnoitiosis (Njenga *et al*., 1995; Shkap *et al*., 2002; Cortes *et al*., 2006, 2007; EFSA, 2010; Basso *et al*., 2013). Enzyme-linked immunosorbent assay (ELISA) and Western blot (WB) tests could be useful tools to detect asymptomatic/sub-clinical cattle for control; however, a gold standard technique has not yet been established. Thus, comparable tests are needed to carry out prevalence studies in different countries to determine the impact of the disease and thus implement effective control programs.

The aim of the present study was to investigate the presence of *B. besnoiti* in cattle from four major areas located in the Eastern and Southeastern Anatolia in Turkey.

Materials and Methods

This study was carried out in Mus, Van, Siirt, and

Diyarbakir provinces between May 2015 and December 2016 (Fig. 1). Blood samples were collected from 450 clinically healthy and asymptomatic cattle (398 females and 52 males). The animals were selected from cattle grazing in the pastures. PrioCHECK®Besnoitia Ab 2.0 ELISA kit (Product No.: 7610530, Version: 2.1_e) (Prionics AG, Schlieren, Switzerland) was used according to manufacturers instructions to detect specific anti-*B. besnoiti* antibodies. The test has a sensitivity of 100%, and specificity of 98.8%. The plates were read in ELISA microplate reader (Bio-Tek Instruments, MicroQuant) at a wavelength of 450 nm. Results were calculated according to the following formula: OD450 of test sample/OD450 of positive control × 100=% positivity. A percentage positivity (PP) ≥23% was considered seropositive.

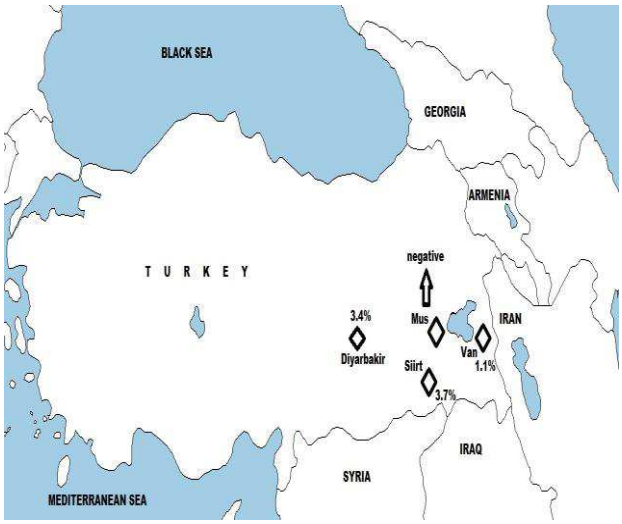

Fig. 1: Map of Turkey; sampling areas along with the prevalence rate of besnoitiosis are presented

Statistical analysis

Pearson's Chi-square test was performed to compare prevalence among gender, age and locality categories. Statistical comparisons were carried out using SPSS 22.0 statistical software (IBM, Unites states). Significance was defined as P<0.05.

Results

As shown in Table 1, 12 (2.7%) of the 450 cattle were seropositive against *B. besnoiti*. There were no statistical differences among counties, age and gender groups for besnoitiosis prevalence. In different age groups, the highest infection rate (3.3%) was recorded 6-8 years in cattle followed by the age group 3-5 years (2.8%). No *Besnoitia* parasites were detected in 1-2 years age group. The prevalence rates were 0%, 3.7%, 3.4%, and 1.1% in Mus, Siirt, Diyarbakir, and Van provinces, respectively (P>0.05). Twelve (3%) females were infected with *B. besnoiti* while the parasite was not found in males.

Table 1: The prevalence rates of *B. besnoiti* infection in cattle according to the locality, gender and age

Variables	Animals tested	ELISA	Seropositive	P-value
	(n)	(n)	(%)	
Age (years)				
1-2	52	-	-	0.441
3-5	248	7	2.8	
6-8	150	5	3.3	
Gender				
Male	52	-	-	0.204
Female	398	12	3	
Locality				
Van	95	1	1.1	0.334
Mus	50	-	-	
Siirt	187	7	3.7	
Diyarbakir	118	4	3.4	
Total	450	12	2.7	

$\chi 2$: Pearson's Chi-square test (P>0.05), and n: number

Discussion

The protozoan parasite *B. besnoiti* has become increasingly recognized as an important cause of economic loss in cattle in endemic regions in the past decade. Bovine besnoitiosis has been reported in many countries with different prevalence rates (Alvarez-Garcia *et al*., 2013). The prevalence rate of 2.7% for besnoitiosis found in this study is significantly lower than that reported for cattle in Spain (90%) (Fernandez-Garcia *et al*., 2010), France (89%) (Alzieu *et al*., 2007), Italy (44.1%) (Rinaldi *et al*., 2013) and Greece (22%) (Papadopoulos *et al*., 2014). In Turkey, there is only one report showing the presence of *B. besnoiti* in cattle. Ocal *et al*. (2016) found antibodies against *B. besnoiti* in 26.6% of cow sera based on ELISA test in Kirikkale province of Turkey.

Currently, the PrioCHECK® kit is recommended as a reliable diagnostic serological test. This method is promising for the characterization of *B. besnoiti*-specific antigens that would not cross-react with *T. gondii* and *N. caninum* (García Lunar *et al*., 2017).

It has been reported that animals with no clinical signs in endemic areas may be a considerable factor in spread of the disease (Frey *et al*., 2013). When the bovine besnoitiosis outbreak emerged in Spain, most of the animals were seropositive (90.5%), but only 43% of seropositive cattle showed at least one clinical signs (Fernandez-Garcia *et al*., 2010). In current study, *B. besnoiti* antibodies were found in 2.7% of animals, although no clinical symptoms were observed in any of the animals. Even in endemic situations, only a few animals of a *B. besnoiti*-infected herd develop characteristic clinical symptom, whereas most are seropositive but remain subclinically infected (Fernandez-Garcia *et al*., 2010; Garcia-Lunar *et al*., 2013). The reasons why only a small percentage of animals develop disease are not yet understood (Frey *et al*., 2013). Serological identification of subclinically infected cattle is important to avoid introduction of infected animals into native herds (Papadopoulos *et al*., 2014).

Gutierrez-Exposito *et al*. (2017) reviewed the

diagnosis and control of bovine besnoitiosis. They suggested that the evaluated ELISAs can be employed in epidemiological studies. Additionally, García Lunar *et al*. (2017) declared that the evaluated three tests (APure-BbELISA, BbSALUVET ELISA 2.0 and new version of PrioCHECK®Besnoitia Ab 2.0) showed good performance and reported 88% sensivity and 98% specificity for PrioCHECK®Besnoitia Ab 2.0 at the cut-off of 23% PP. Moreover, the confirmation of positive ELISA results with a follow-up WB test is no longer necessary because of the high level of specificity (García Lunar *et al*., 2017).

Previously, two main limitations such as false negative (Fernández-García *et al*., 2010; García-Lunar *et al*., 2013; Gutiérrez-Expósito *et al*., 2017) and false-positive results (Schares *et al*., 2011; Nasir *et al*., 2012; Uzëda *et al*., 2014) have been shown during various serological studies. Thus, the verification of positive or negative ELISA data using a WB test is advised in special cases, such as with inconclusive results, cattle prior to entry into herds free of the disease and precious animals previous to a selective culling (García-Lunar *et al*., 2013).

Schares *et al*. (2011) evaluated the first version of this commercial test (PrioCHECK®Besnoitia Ab), and a specificity of 94.3%-96.8% was recorded depending on the applied cut-off PP of 10%-20%, respectively. In Greece, using the same version of ELISA kit at cut-off PP 20% showed that 22% of cows were seropositive to *B. besnoiti* without the confirmation with WB (Papadopoulos *et al*., 2014). In Italy, the individual animal prevalence was found 44.1% using the PrioCHECK®Besnoitia Ab 2.0 by Rinaldi *et al*. (2013). However, the authors did not report the PP value used in their study. A total of 101 (13.17%) samples showed a positive reaction in ELISA (PrioCHECK®Besnoitia Ab 2.0; cut-off: (PP) ≥15) in Switzerland (Basso *et al*., 2013). Nasir *et al*. (2012) also reported *B. besnoiti* antibodies by the same ELISA kit (cut-off threshold: 15 PP) in South Australia; however these ELISA results were not confirmed with WB or IFAT tests.

From the results of the above studies it is clear that the different cut-off values used in studies significantly affect the specificity and sensitivity of the ELISA kits and prevalence rate of besnoitiosis. Thus, in this study, some higher restrictive cut-off value (23 PP) was used (Schares *et al*., 2011; Nasira *et al*., 2012; Basso *et al*., 2013; Papadopoulos *et al*., 2014). In the present study, a total of 12 samples showed a positive reaction in ELISA. However, besnoitiosis has not yet been reported with typical clinical signs in cattle in Turkey.

Consequently, it is understood that both bovine besnoitiosis have many aspects that need to be clarified. Effect of the disease still remains unknown in Turkey and needs further investigation. The presence of the disease in cattle and probably goats in other geographical regions of Turkey and risk factors affecting spread of the parasite should be investigated. In addition, we need to raise veterinarians awareness of the issue so that clinical cases are not neglected.

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Conflict of interest

The authors declare that there is no conflict of interest.

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