

Seroepidemiological investigation of brucellosis in sheep abortions in Kars, Turkey

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Abstract This study was undertaken to investigate the seroprevalence of brucellosis in unvaccinated sheep from the flocks having previous abortion cases in Kars and around, Turkey and to compare the efficacy of each serological test used. Four hundred serum samples collected from 16 different flocks of sheep having a history of abortions in Kars and its surrounding area in Turkey were examined for the presence of antibodies raised against *Brucella* using Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Rivanol Agglutination Test (RAT) and Complement Fixation Test (CFT). All animals were unvaccinated against *Brucella*. Of the serum samples tested, 147 (%36.7), 142 (%35.5), 139 (%34.75) and 135 (%33.75) were found positive by SAT, RAT, RBPT and CFT, respectively. No statistically significant difference was found between the serological tests used ($p>0.05$). It is concluded from this study that brucellosis continues to be an important problem for ovine abortions and poses a risk both for human and other animals in this area. Therefore, adequate intervention measures should be implemented to

control and eradicate brucellosis. In addition, if conventional serological tests are used at least two tests, RPBT for screening and CFT for the confirmation of the positive samples are preferable, should be used in parallel for detection of brucellosis effectively.

Keywords Brucellosis · Serological tests · CFT · SAT · RBPT · RAT

Introduction

Brucellosis is a zoonotic infection that can be transmitted from infected animals and/or via their products such as milk and dairy products to humans (Arda et al. 1997). *Brucella* spp. cause reproductive system disorders such as abortion and infertility in various animals including sheep (Alton et al. 1988). It also causes serious infections in humans. Although brucellosis is now eradicated in most developed countries it is still an important problem in some part of the world (Iyisan et al. 2000; Benkirane 2006). Due to its significance both for public and animal health and its economic consequences, it is important to take necessary steps to control and eradicate brucellosis from endemic areas (Minas 2006).

Brucella melitensis is an important etiological agent of brucellosis in ovine abortions (Arda et al. 1997; Minas 2006). Conventional isolation of the bacterial species is required for the definitive diagnosis of brucellosis and is taken as the ‘Gold Standard’.

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However it takes time, is labour intensive and expensive to perform and due to its zoonotic potential and it is not always possible to isolate etiological agent from infected animals (Erganis et al. 2002; McGiven et al. 2003). Therefore, the presumptive diagnosis is mainly based upon serological testing that detects antibody response raised against *Brucella* antigens (Bercovich et al. 1998; Jacques et al. 1998). A number of serological tests such as Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Rivanol Agglutination Test (RAT), complement-fixation test (CFT), Agar Gel Diffusion Technique (AGIT) and ELISA are widely used for this purpose (Diaz-Aparicio et al. 1994; Bercovich et al. 1998; Omer et al. 2001; Erdenebaatar et al. 2002; McGiven et al. 2003; Nielsen et al. 2005). Each test has advantage(s) and/or disadvantages over each other in their ability to detect *Brucella* antibodies. Since diagnosis and mass testing programmes of brucellosis in animals mainly rely on presumptive identification by the serological tests, the efficacy and accuracy of the test employed has great impact on the success of an intervention programme (Minas 2006; Nielsen et al. 2005). The CFT has a high specificity and sensitivity in naturally infected animals and is widely used as a confirmatory test (Omer et al. 2001; Nielsen et al. 2005). It is also suggested as prescribed test for brucellosis in small ruminants by the OIE (OIE 2000).

The aim of this study was to investigate the seroprevalence of brucellosis in sheep belonging to the flocks having a history of abortion and no vaccination against *Brucella* using RBPT, SAT, RAT and CFT in Kars and its surrounding area, Turkey and to compare the efficacy of each serological test employed.

Materials and methods

Serum samples

In this study, a total of 400 sera obtained from 16 different sheep flocks with a history of abortion and no vaccination against *Brucella* from various locations in Kars which is located on the border in north-eastern part of Turkey were examined for the presence of *Brucella* antibodies. The animals were selected randomly from the sheep flocks that had previous abortion cases.

Serological tests

All the serum samples collected from the sheep were tested by RBPT, SAT, RAT and CFT. Positive and negative sera that were obtained from Institut Pourquier-Montpellier (France) were also included for each test throughout the study to avoid misinterpretations. *Brucella abortus* whole-cell antigen that was purchased from Pendik Veterinary Control and Research Institute (PVCRI), Turkey was used for RBPT, SAT and RAT assays.

RBPT was performed according to procedures described by Alton et al. (1988). Briefly, an equal volume (30 μ l) of the antigen and serum was mixed on a clean glass slide and shaken gently. Formation of agglutination after 4 min was accepted as positive result.

Two-fold dilutions of the sera were prepared from 1/5 to 1/320 in order to perform the SAT. An equal volume of test antigen purchased from PVCRI was added to each serum dilution and the tubes were incubated at 37°C for 18–24 h. The results were interpreted as described previously (Alton et al. 1988). Since the animals were unvaccinated against *Brucella*, a positive titre of $\geq 1/20$ was used to reflect seropositivity according to Arda et al. (1997).

RAT was carried out according to procedures described by the Ministry of Agriculture Brucellosis Control Programme Guidelines, Turkey (Anonym 1990). Briefly, the sheep sera were subjected to 0.04% rivanol and then two-fold dilutions between 1/5 and 1/320 were prepared. $\geq 1/10$ was interpreted as positive test result.

CFT was performed as described by Alton et al. (1988). *Brucella abortus* antigen used for the CFT assay was obtained from Institut Pourquier-Montpellier (France). The sera were diluted in veronal buffer (VB) and inactivated at 62°C for 30 min to prevent anti-complementary activity. A positive titre of 1/5 ++ or higher (100% inhibition of haemolysis) was interpreted as seropositive. The sera having anti-complementary activity were subjected to 5% bovine serum albumine fraction V in VB at 37°C for 30–60 min and then inactivated 62°C for 30 min. After that, the CFT steps were followed as above.

Statistical analysis

χ^2 test was used in order to compare the results of serological tests using SPSS for Windows Release 10.0 (SPSS inc 1989–1999) statistical package.

Results

Of the 400 sheep sera examined in this study, 135 (33.75%), 139 (34.75%), 142 (35.5%) and 147 (36.7%) were found positive for *Brucella* antibodies by CFT, RBPT, RAT and SAT, respectively. The serological test results are summarized in Table 1. Of the 16 different flocks of sheep with a history of abortions, 14 (87.5%) were found seropositive in the current study. Determination of the high prevalence of brucellosis in sheep both individually and at the flock level suggests that brucellosis constitutes a serious risk both for animal and human health in the area. The presence of brucellosis in animals including sheep has previously been reported from this area by various researchers (Seyda et al. 1997; Iyisan et al. 2000). Seyda et al. (1997) included 1580 sheep sera collected from the same region in their study and determined 37% seropositivity, which agrees with the results of our study. Iyisan et al. (2000) reported that brucellosis in is highly endemic in cattle and sheep populations (15% in sheep and 20.8% in cattle) this area at the flock/herd level. All sheep flocks examined in this study had previous abortion histories and all animals were not vaccinated against brucellosis. Although bacterial isolation was not performed from seropositive ewes in the present investigation and the serological test results are usually considered as an

indication of brucellosis and provide presumptive identification (Bercovich et al. 1998). Therefore, it may be stated from the findings of this study that brucellosis plays an important etiological role in ovine abortions in this area. It also constitutes a risk for humans due to zoonotic nature of brucellosis, which can be transmitted to humans by direct contact with infected animals or via their products such as milk and dairy products made from raw and/or unpasteurized milk. Therefore, effective intervention measures should be implemented to control and eradicate brucellosis in this area to protect both human and animal health. Intervention measures involve immunization of the susceptible animals and strict control of animal movements on the borders (Minas 2006).

Discussion

In the present study, 136 sera were found positive by both RBPT and SAT however, 8 sera found negative by RBPT was positive by SAT (see also Table 1). In addition, SAT performed slightly better (147 positive *versus* 139) than RBPT for the detection of *Brucella* antibodies, which is in agreement with previous reports (Mahajan and Kulshreshtha 1991). These variations may be due to the ability of each test to detect different antibody classes; The RBPT which is

Table 1 Comparison of the serological tests for detection of *Brucella* antibodies in sera obtained from different sheep flocks with abortion histories

Serological Tests ¹	RBPT		SAT		RAT		CFT	
	139 + +	261 - -	147 + +	253 - -	142 + +	258 - -	135 + +	265 - -
RBPT	+		136 (92.5)	14	138 (97.1)	12	133 (98.5)	17
	-		8	242 (95.6)	7	243 (94.1)	4	246 (92.8)
SAT	+	136 (97.8) ²	8		131 (92.2)	15	127 (94.0)	16
	-	14	242 (92.7)		7	247 (95.7)	9	248 (93.5)
RAT	+	138 (99.2)	7	131 (89.1)	7		134 (99.2)	10
	-	11	243 (93.1)	15	247 (97.6)		4	252 (95.0)
CFT	+	133 (95.6)	4	127 (86.3)	9	134 (94.3)	4	
	-	17	246 (94.2)	16	248 (98.0)	10	252 (97.6)	

¹, a total of 400 sheep sera were tested.

², numbers in parentheses represent the percentage of test result

+, positive test result.

-, negative test result.

widely used as a screening test for brucellosis performs better to detect IgG and the SAT can detect immunoglobulins of both IgG and IgM classes.

As seen in Table 1, 95.6% of the sera determined as positive by RBPT were also found positive by CFT. 86.3% of the sera found positive by SAT were also positive by CFT, whereas 94% of the CFT positive sera were positive by SAT. In addition, 98% of the SAT negative serum samples were also found negative by CFT. 94.3% of the RAT positive sera were CFT positive whereas 99.2% of the CFT positive samples were positive by the RAT. This shows that the sensitivity of RAT and CFT is almost similar as also reported by Nicoletti (1969).

Diagnostic efficiency between the serological tests used in this study showed little difference but this was not statistically significant ($p > 0.05$). The tests employed here were able to detect brucellosis in a range of 33.7 to 36.7%, which supports the view that a single test is often inadequate to effectively detect brucellosis (Bercovich et al. 1998; Mahajan and Kulshreshtha 1991). Therefore, at least two serological tests should be combined in order to detect brucellosis most effectively in an infected sheep flock. The use of a simple serological test of high sensitivity such as RBPT in combination with a confirmatory test of high specificity such as CFT is regarded as being the most effective way for the conventional detection and implementation of successful control programmes for brucellosis (Omer et al. 2001).

It is considered that a sensitive test will determine the most true- positive animals and a specific test will determine the most true-negative animals (Bercovich et al. 1998). The CFT is usually employed as the confirmatory test by many researchers for the detection of brucellosis (Benkirane 2006) and considered to be 100% specific in non-vaccinated animals (Jacques et al. 1998; Omer et al. 2001). By the CFT, 135 (33.75%) of the sheep sera were determined positive and 265 (66.25%) were determined negative. Of the CFT positive sera, 99.2%, 98.5% and 94% were determined as positive by RAT, RBPT and SAT, respectively (Table 1). This shows that sensitivity of the CFT, RAT and RBPT was almost similar to each other but that of SAT was lower relative to the CFT reactivity. Our results almost paralleled with that of Jimenez de Bagues et al. (1992) for CFT and RBPT and contradicted with those of Bercovich et al. (1998) and Jacques et al. (1998) for CFT, SAT and RBPT.

However, Jacques et al. (1998) reported that they only received different results between CFT and RBPT when ewes were challenged with higher dose of bacteria; no significantly different results were observed when the animals were given lower doses. Regarding the specificity of the assays employed in relation to CFT; of the 265 CFT negative sera, 95%, 93.5% and 92.8% were also negative by RAT, SAT and RBPT, respectively. These three serological tests have lower specificity values than CFT although RAT is slightly better in specificity than SAT and RBPT. The reason for this discrepancy may be due to interference by non-specific antibodies resulting from cross-reacting antigens (Nielsen et al. 2005).

In conclusion, the high prevalence of brucellosis in unvaccinated sheep flocks having history of abortions suggests that brucellosis is endemic in the area and plays an important role for ovine abortions. Therefore, adequate intervention measures should be implemented to control and eradicate brucellosis for the prevention of both human and animal health. In addition, if conventional serological tests are used at least two tests, RBPT as rapid screening and CFT as confirmation of the positive samples are preferable, should be combined for the effective detection of brucellosis.

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