TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Case Report

Turk J Vet Anim Sci
(2018) 42: 665-668
© TÜBİTAK
doi:10.3906/vet-1803-54

The first-time isolation of *Brucella canis* from two aborted bitches in a kennel in Turkey

Ahmet Murat SAYTEKİN[1,*], Mustafa Sencer KARAGÜL[2], Buket EROĞLU[1], Gülseren YILDIZ ÖZ[1], Gülnaz YILMAZBAŞ MECİTOĞLU[3], Özge YILMAZ[4]

[1]Brucella Reference Diagnosis Laboratory, Pendik Veterinary Control Institute, İstanbul, Turkey
[2]Vocational School of Equine Science, Kocaeli University, Kocaeli, Turkey
[3]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Uludağ University, Bursa, Turkey
[4]Department of Microbiology, Faculty of Veterinary Medicine, Uludağ University, Bursa, Turkey

Received: 15.03.2018 • **Accepted/Published Online:** 29.11.2018 • **Final Version:** 10.12.2018

Abstract: *Brucella canis* is defined as the specific agent that is responsible for canine brucellosis. In this study, *B. canis* was isolated and identified from the clinical materials belonging to 2 dogs kept in a breeding kennel in Turkey and brought to the researchers' clinic with abortion complaints. With this study, for the first time ever, *B. canis* isolation and identification was made from dogs that aborted in a kennel. This study emphasizes the necessity to consider and evaluate dog abortions that occur for unspecified reasons within the scope of brucellosis.

Key words: Abortion, *Brucella canis*, isolation, kennel

1. Introduction

Brucella canis is a facultative intracellular pathogen that preferentially infects members of the family Canidae (1). Although canine brucellosis caused by *B. canis* has been reported worldwide (2), few human cases have been reported so far (1). In Turkey, as well, there are few serological notifications of the disease in humans (3,4). Karaşahin et al. (4) isolated the specific agent from the endocarditis case of a patient. In Turkey, there are many instances of serological and molecular diagnosis of *B. canis* in dogs (5–7), and it was once isolated from lymph nodes (5). This study has investigated the existence of brucellosis agents in two aborted bitches in a kennel.

2. Case history

Due to the increase in the instances of dog breed pregnancies resulting in abortion, the owner of the breeding kennel consulted the Uludağ University Veterinary Faculty's Obstetrics and Gynecology Department's clinic in July 2015. According to the history, a Pug dog named Lady (Case 1) had aborted 1 month ago and a dog named Gamze (Case 2) had aborted 9 days ago. The dogs had aborted at about 50–51 days after the last mating with 22-day interval. Case 1 was a 2-year-old nulliparous bitch and Case 2 was a 3-year-old multiparous bitch (previously gave birth twice). It was stated that 4 different dogs had aborted within the last 2 months in the kennel.

Clinical examinations, such as body temperature, heart and respiration rates, capillary filling time, and lymph node palpation, of both referred bitches were performed and revealed no abnormality. With the suspicion of brucellosis, vaginal swab and blood samples were taken under sterile conditions. The blood samples taken for serological examination were cooled to 4 °C and blood samples were centrifuged at 2000 × *g* for 20 min at 4 °C. The obtained sera were sent to the Pendik Veterinary Control Institute (PVKE) with other samples under cold chain conditions.

The vaginal swabs and blood samples belonging to the two dogs were used for bacterial isolation. The samples were inoculated on Farrell's and CITA media. Besides these selective media, serum dextrose agar medium was used as a nonselective medium. Therefore, samples were inoculated in parallel on 3 different media separately. They were incubated on plates under 6% CO_2 at 37 °C for 6–8 days. Colony formation was checked on a daily basis (8,9). On the 4th day of inoculation, Gram staining of honey-colored transparent colonies, typically 1–2 mm in diameter, was carried out for bacterial identification at the genus level. The typical gram-negative coccobacillus image was observed after the staining process. Agglutination tests with *Brucella* monospecific R antiserum (Veterinary Laboratory Agency; PA0071), with *Brucella* polyclonal A-M antiserum (PVKE; 2008-1), and with 0.1% acriflavine (Sigma, A-8126) and additionally catalase (Merck,

* Correspondence: msaytekin@yahoo.com.tr

108597), oxidase (Merck, 113300), and urease tests were performed (8).

For identification at species level, several tests were conducted, including the carbon dioxide requirement test; hydrogen sulfide (H_2S) gas production test; growth tests in the media containing thionine (Sigma, T3387), basic fuchsin (Merck, 115937), safranin (Sigma, S2255), penicillin (Fluka, 13750), streptomycin (Sigma, S-6501), and erythritol (Sigma, E7500) at routine test dilutions; and Tbilisi (Tb) (PVKE; FTb-01-07), Izatnagar (Iz) (PVKE; FIz-05-07), and R/C (PVKE; FR/C-08-07) phage tests and agglutination tests with *Brucella* monospecific A and M antisera (PVKE; 2010-1). The media were incubated under 6% CO_2 at 37 °C for 5 days. The CO_2 requirement test was performed under aerobic conditions (8,9).

For molecular diagnosis from the culture, DNA isolation was performed by boiling. For molecular identification at the genus level, B4 (forward) and B5 (reverse) primers were utilized according to the modified version of the method suggested by Queipo-Ortuno et al. (10). A 223-bp amplification product from the gene encoding 31 kDa of *Brucella* cell surface salt extractable protein (BCSP) was obtained using these 2 primers. For molecular identification at species level, the method used by Mayer-Scholl et al. (11) was applied to the suspicious bacteria cultures.

The sera were analyzed by rapid slide agglutination test (S-RSAT) with Rose Bengal-stained antigen (PVKE; RBPT/01/15) prepared from the *Brucella abortus* S99 smooth strain and rapid slide agglutination test (R-RSAT) with Rose Bengal-stained antigen prepared from the *B. canis* M(-) rough strain. The serum agglutination test (S-TAT) with unstained antigen (PVKE; TAT/01/15) prepared from the *B. abortus* S99 smooth strain and modified mercaptoethanol tube agglutination tests (R-2ME-TAT) with unstained antigen prepared from the *B. canis* M(-) rough strain were also carried out (8).

3. Results and discussion

The results of the agglutination tests with polyclonal *Brucella* R antiserum and acriflavine, as well as the results of catalase, oxidase, and urease tests, were found to be positive. However, the result of the agglutination test with *Brucella* polyclonal A-M antiserum was found to be negative. According to these results, *Brucella* spp. in rough form was isolated and identified from the vaginal swab (isolate 1) and full blood (isolate 2) of Case 2 and from only the vaginal swab (isolate 3) of Case 1. Therefore, *Brucella* spp. isolation and identification was made from the samples of both dogs. Bacterial isolation is regarded as the gold standard for the diagnosis of brucellosis from clinical samples (8,9). It was reported that using Farrell's and CITA media, which are recommended as selective media, simultaneously increases isolation sensitivity to the highest level and makes the diagnosis of the agent easier (9). In this study, as well, Farrell's and CITA media were used concurrently as recommend in the related literature. For this reason, the use of three media, consisting of two different selective media and one basal medium, contributed to the isolation sensitivity.

The isolates were identified as *B. canis* as a result of the tests performed on the isolated *Brucella* isolates for species-level identification. Six different reference strains, which were used as controls, were identified correctly at species level, as shown in the Table. Researchers from different countries have reported the isolation and identification of *B. canis* from clinical samples taken from dogs (12,13). In a study carried out in Turkey, isolation of *B. canis* from lymph nodes (5) was also reported. In this study, *B. canis* was isolated for the first time ever from samples belonging to two dogs that had aborted earlier (9 days and 1 month ago) in a breeding kennel.

As a result of the rapid slide agglutination tests on sera, a negative result was obtained through S-RSAT and a positive result was obtained through R-RSAT. As a result of serum agglutination tests applied on sera, a negative result was obtained through S-TAT and a positive result was obtained through R-2ME-TAT. Researchers have performed serological tests for the serological diagnosis of the disease by using antigens prepared from rough strains, and many serologically positive findings have been reported in Turkey (3,7,14) and in various other countries in the world (13). Researchers have also performed serological tests with blood sera using smooth antigens in some conducted studies, which were related to *B. canis* originating from dog brucellosis cases, and they reported the test results as negative (4,14). In this study, in accordance with bacterial isolation, the disease was also diagnosed serologically by confirming its nonsmooth antigenic structure.

As a result of the genus-specific Bcsp31 PCR, specific bands of 223 bp in length were determined for isolates and reference strains, and all strains were identified as *Brucella* spp. in accordance with bacterial isolation, as shown in Figure 1.

The developed multiplex PCR test (Bruce-ladder) provides fast and simple identification at the species level. Researchers reported that the only disadvantage of the test is that it cannot differentiate biotypes and detects some *B. canis* isolates erroneously as *Brucella suis* (15). In our study, although appropriate band profiles were detected with reference strains, a *B. suis* profile was obtained from isolates, as shown in Figure 2. Therefore, it is seen that species-specific molecular identifications of isolates do not conform to conventional bacterial identifications at species level. The multiplex PCR results of isolates have been

Table. Bacterial identification test results.

Samples	Identification test results at species and biotype level														
	CO_2 requirement	H_2S production	Growth in thionine medium	Growth in basic fuchsin medium	Growth in safranin medium	Growth in streptomycin medium	Growth in penicillin medium	Growth in erythritol medium	Phage tests (routine test dilution)			Antisera			Results
									Tbilisi	Izatnagar	R/C	A	M	R	
1	-	-	W	+	+	-	+	+	-	-	+	-	-	+	B
2	-	+	+	-	-	-	-	+	-	+	-	+	-	-	C
3	-	-	+	-	-	-	+	+	-	-	+	-	-	+	B
4	-	-	+	-	-	-	-	+	-	-	+	-	-	+	B
5	+	-	+	-	-	-	-	+	-	-	+	-	-	+	D
6	-	-	-	-	-	+	-	+	-	+	-	-	+	-	E
7	+	+	+	+	+	-	+	+	+	+	-	+	-	-	F

1- Isolates, 2- *B. suis* 1330, 3- *B. canis* RM6/66, 4- *B. canis* M(-), 5- *B. ovis* 63/290,
6- *B. melitensis* Rev-1, 7- *B. abortus* Tulya, W- weak growth, B- *B. canis*, C- *B. suis* biotype 1, D- *B. ovis*, E- *B. melitensis* Rev-1, F- *B. abortus* biotype 3.

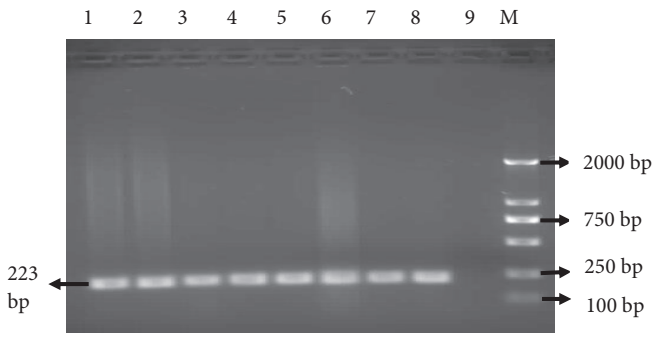

Figure 1. Bcsp31 PCR results. 1- *B. canis* RM 6/66, 2- *B. canis* M(-), 3- *B. ovis* 63/290, 4- *B. suis* 1330, 5- isolate 1, 6- isolate 3, 7- *B. abortus* Tulya, 8- *B. melitensis* Rev-1, 9- negative control, M- marker, 2000 bp.

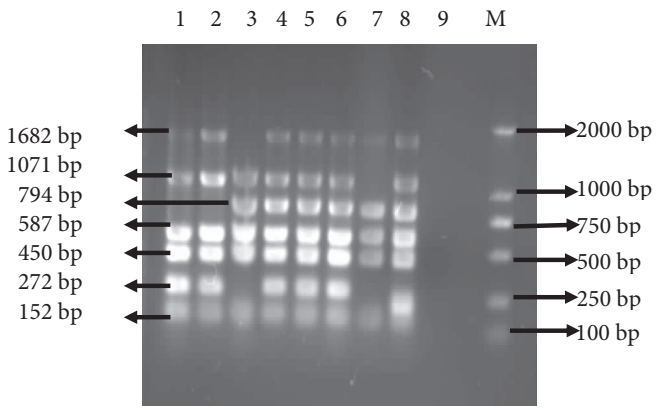

Figure 2. DNA amplification results at species level. 1- *B. canis* RM 6/66, 2- *B. canis* M(-), 3- *B. ovis* 63/290, 4- *B. suis* 1330, 5- isolate 1, 6- isolate 3, 7- *B. abortus* Tulya, 8- *B. melitensis* Rev-1, 9- negative control, M- marker, 2000 bp.

evaluated as one of the erroneous results that stem from the nature of the test as indicated by previous investigators, in which some *B. canis* isolates may be detected as *B. suis*.

As a result, this study has evidenced the existence of the disease in Turkey more strongly than previous studies and showed the importance of the consideration and evaluation of dog abortions within the scope of brucellosis. The threat of the disease for dogs' and humans' health was also emphasized by proving the existence of the agents in the fluids of abortion even 1 month later from a case of abortion. As control measures, regular disease scans should be conducted on male and female breed dogs in kennels, dogs imported to Turkey should be examined for the disease, and awareness should be built in society by establishing a legal infrastructure for the fight against canine brucellosis in Turkey.

References

1. Viana MVC, Wattam AR, Govil Batra D, Boisvert S, Brettin TS, Frace M, Xia F, Azevedo V, Tiller R, Hoffmaster AR. Genome sequences of three *Brucella canis* strains isolated from humans and a dog. Genome Announc 2017; 5: 1-2.
2. Holst BS, Löfqvist K, Ernholm L, Eld K, Cedersmyg M, Hallgren G. The first case of *Brucella canis* in Sweden: background, case report and recommendations from a northern European perspective. Acta Vet Scand 2012; 54: 1-9.
3. Diker S, İstanbulluoğlu E, Ayhan H, Soysal GA. Sero-survey of *Brucella canis* infections in man at Bursa district. Mikrobiyol Bült 1984; 18: 203-207 (in Turkish with abstract in English).
4. Karaşahin Ö, İba-Yılmaz S, Aslan MH, Çelik N. Infective endocarditis due to *Brucella canis*: a case report. Klimik Derg 2016; 29: 138-141 (in Turkish with abstract in English).
5. Aras Z, Uçan US. Detection of *Brucella canis* from inguinal lymph nodes of naturally infected dogs by PCR. Theriogenology 2010; 74: 658-662.
6. Aras Z, Taşpınar M, Aydın İ. A novel polymerase chain reaction to detect *Brucella canis* in dogs. Kafkas Univ Vet Fak 2015; 21: 169-172.
7. Yumuşak N, Polat PF, Erdenliğ Gürbilek S, Şen ÇC, Yılmaz R. A comparison of the performance of exfoliative cytopathology, polymerase chain reaction (PCR), culture and ELISA in the detection of *Brucella canis*. Harran Üniversitesi Veteriner Fakültesi Dergisi 2017; 6: 51-56.
8. Alton GG, Jones LM, Angus RD, Verger JM. Techniques for the Brucellosis Laboratory. Paris, France: Institut National de la Recherche Agronomique; 1988.
9. World Organisation for Animal Health. Terrestrial Manual. Chapter 2.1.4. Brucellosis. Paris, France: OIE; 2016.
10. Queipo-Ortuno MI, Morata P, Ocon P, Manchado P, Colmenero JD. Rapid diagnosis of human brucellosis by peripheral blood PCR assay. J Clin Microbiol 1997; 35: 2927-2930.
11. Mayer-Scholl A, Draeger A, Golner C, Scholz HC, Nockler K. Advancement of a multiplex PCR for the differentiation of all currently described *Brucella* species. J Microbiol Methods 2010; 80: 112-114.
12. Keid LB, Chiebao DP, Batinga MCA, Faita T, Diniz JA, Oliveria TMFS, Ferreira HL, Soares RM. *Brucella canis* infection in dogs from commercial breeding kennels in Brazil. Transbound Emerg Dis 2017; 64: 691-697.
13. Sanchez-Jimenez MM, Ortiz-Roman LF, Castrillon-Salazar LL, Giraldo-Echeverri CA, Olivera-Angel M. Application of a polymerase chain reaction test for the detection of *Brucella canis* from clinical samples of canines and humans. Rev Colomb Cienc Pec 2014; 27: 3-11.
14. Sayan M, Erdenlig S, Stack J, Kilic S, Guducuoglu H, Aksoy HY, Baklan EA, Etiler N. A serological diagnostic survey for *Brucella canis* infection in Turkish patients with brucellosis-like symptoms. Jpn J Infect Dis 2011; 64: 516-519.
15. Lopez-Goni I, Garcia-Yoldi D, Marin CM, De Miguel MJ, Munoz PM, Blasco JM, Jacques I, Grayon M, Cloeckaert A, Ferreira AC et al. Evaluation of a multiplex PCR assay (Bruce-ladder) for molecular typing of all *Brucella* species and of the vaccine strains. J Clin Microbiol 2008; 46: 3484-3487.