German Journal of Veterinary Research

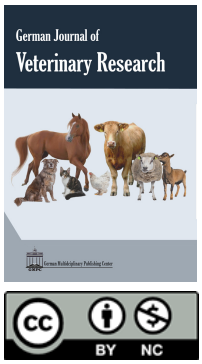
eISSN:2703-1322

Short communication

The first report of *Brucella melitensis* biovar 2 strain isolated from cattle in Turkey

Kadir Akar[1*] **and Gülseren Yıldız Öz**[2]

[1] Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Van Yuzuncu Yıl University, 65090 Van, Turkey
[2] NRL for Brucellosis, Pendik Veterinary Control Institute, 34890 Istanbul, Turkey



Article History:
Received: 03-Mar-2023
Accepted: 10-Apr-2023
***Corresponding author:**
Kadir Akar
kadirakar87@gmail.com

Abstract

Bovine brucellosis is an infectious zoonotic disease of great impact on animal welfare and has significant economic implications on livestock farm worldwide. The disease is caused primarily by *Brucella abortus* (*B. abortus*), while *B. melitensis* is less common, and *B. suis* infection is rare. *B. melitensis* is the most common causative agent of brucellosis in small ruminants and humans. Although the main host of *B. melitensis* is considered to be small ruminants, this bacterium is also present in large ruminants. Despite brucellosis has been eradicated in many European countries, it is still endemic in Mediterranean countries and Turkey. The most prevalent *Brucella* species in the Mediterranean basin and Turkey is *B. melitensis* biovar (bv) 3. Previous studies have reported that *B. melitensis* bv2 is quite low in Turkey. This is the first study to isolate *B. melitensis* bv2 from cattle in Turkey. The strains were characterized using classical biotyping methods and then were molecularly confirmed. Multilocus variable number tandem repeat analysis (MLVA-16) typing of the strains revealed a novel genotype (1-5-3-13-3-2-3-2-4-41-8-5-4-3-3-7), which matches the Multilocus sequence typing (MLST) profiles in the database of ST8 (3-2-3-2-1-5-3-2-8). These results indicate that *B. melitensis* bv2 can easily infect cattle and this has to be considered in the epidemiology and control of bovine brucellosis. Circulating the highly pathogenic *B. melitensis* bv2 in cattle farms is of public health concern.

Keywords: *Brucella melitensis* bv2; Turkey; Cattle; Brucellosis

Citation: Akar, K. and Öz, G. Y. 2023. The first report of *Brucella melitensis* biovar 2 strain isolated from cattle in Turkey. Ger. J. Vet. Res. 3 (2):11-15. https://doi.org/10.51585/gjvr.2023.2.0053

Introduction

Brucellosis is a very common zoonotic disease, which is endemic in the Middle East and the Mediterranean basin, including Turkey (Akar and Erganis, 2022; Wareth et al., 2019). Brucellosis has a significant public health importance and economic impacts on farm animals associated with reproductive problems, such as abortion, retained placenta, and infertility. Although the primary hosts for *B. melitensis* are small ruminants, it can be transmitted to cattle and wild ruminants (Jamil et al., 2022). *B. melitensis* biovar (bv) 3 is the most common strain circulating in Mediterranean countries, including Turkey (Wareth et al., 2022). In the brucellosis serosurvey study in Turkey in 2011, the prevalence of *B. melitensis* bv3 in cattle and sheep was 7.8% and 22.5%, respectively (Yumuk and O'Callaghan, 2012). Thus, the conjunctival mass vaccination strategy against *Brucella* was implemented. Since then, every year, several regional veterinary institutes regularly send *Brucella* spp. isolates to the National Reference Laboratory for Brucellosis to collect epidemiological data via biotyping. Previous studies in Turkey showed that *B. melitensis* bv2 was isolated from sheep and goats (Karagul et al., 2017); however, this biovar is quite limited. In this study, we identified *Brucella* strains recovered from two cattle in different years confirmed as *B. melitensis* bv2.

Materials and methods

Bacterial strains

Two *Brucella* strains were investigated in the current study. Both were recovered from cases of abortion in two cattle farms in Turkey. The farms were regularly investigated and archived by the National Reference Laboratory for Brucellosis to create an epidemiological database since 2006. One strain was isolated from cattle in Olur district, Erzurum province in 2018 (2018/83–63), and one from Mersin province Tarsus district in 2021 (2021/25–2).

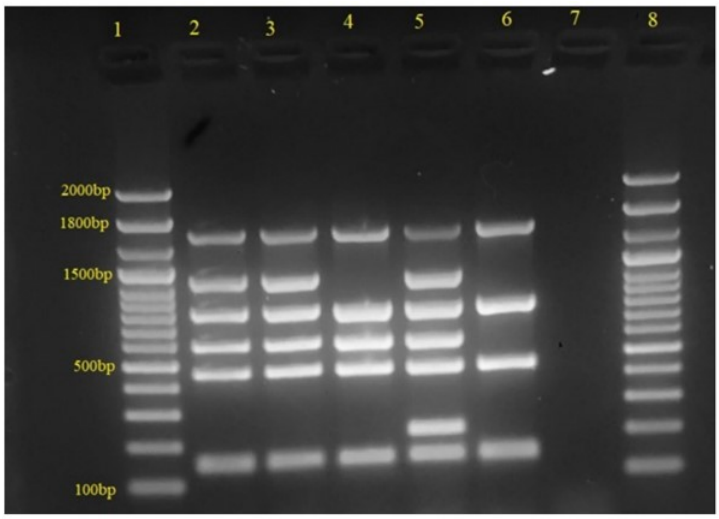


Figure 1: Bruce-Ladder PCR showing molecular confirmation of the investigated strains. Lanes 1 and 8: markers, lanes 2 and 3: Strains 2018/83-63 and 2021/25-2 isolated from cattle shows typical *B. melitensis* profile, lane 4: Positive control (*B. abortus* Tulya), lane 5: Positive control (*B. melitensis* Rev-1 vaccine strain), lane 6: Positive control (*B. abortus* S-19 vaccine strain) lane 7: negative control.

***Brucella* biotyping and molecular confirmation by Bruce-Lader PCR**

Both isolates were identified by standard bacteriological methods, such as growth characteristics in media containing thionine (20 μg/mL), basic fuchsin (20 μg/mL), penicillin (5 IU/mL), streptomycin (2.5 μg/mL), i-erythritol (1 mg/mL). The ability of bacteria to produce H_2S, growth in the presence of CO_2, agglutination with monospecific antisera (anti-A, anti-M), and susceptibility to *Brucella* phage at 104 routine test dilution (RTD) (Tbilisi, Izatnagar, R/C) were also investigated as previously described (Alton et al., 1988). Confirmation and molecular typing of *Brucella* spp. using multiplex PCR (Bruce-ladder) were performed using the method described previously (Mayer-Scholl et al., 2010). Briefly, genomic DNA was extracted using a commercial extraction kit (High Pure FFPET DNA Isolation Kit, 06650767001; Roche Diagnostics, Mannheim, Germany). PCR analysis was performed using a 25 μL-reaction mix containing 2× Qiagen Multiplex Master Mix (Qiagen, Hilden, Germany), 0.2 μM of each primer, and 1 μL the DNA template. Amplification was performed using a thermal cycler (Palm Cycler, Corbett, USA), with the following conditions: initial denaturation at 95°C for 15 min; followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 90 s, and extension at 72°C for 180 s. Final extension at 72°C for 10 min, and a holding temperature of 4°C was applied.

Multilocus variable number tandem repeat analysis and Multilocus sequence typing analysis

Multilocus Variable-Number Tandem Repeat Analysis (MLVA) of the two *B. melitensis* isolates was performed as described previously (Le Flèche et al., 2006; Garofolo et al., 2013). GeneMapper software version 6 was used to analyze the data. *B. melitensis* 16 M reference strain was used as positive control strain. The alleles and repetitive numbers for each locus were obtained from the MLVA database (http://mlva.u-psud.fr). Multilocus sequence typing (MLST) was performed as previously described (Whatmore et al., 2007), and raw data were evaluated using GeneStudio software. The data were loaded into the MLST database (https://pubmlst.org/Brucella/) to determine the allele and ST numbers.

Results and discussion

After five days of incubation with 5% CO_2, *Brucella*-like, translucent, and pale honey-colored colonies identified as Gram-negative isolates were observed. Table 1 presents the biotyping results of the two isolates. Both isolates were identified as *B. melitensis* bv2. Next, Bruce-ladder PCR confirmed the identity of both strains using the purified DNA extracts from the colonies. The six fragments of 1682, 1071, 794, 587, 450, and 152 bp were successfully amplified and exhibited the same amplification pattern as that of the reference strain (Figure 1). According to MLVA-16, the profile of 2018/63-8 (Erzurum) (1-5-3-13-3-2-3-2-4-41-8-6-4-3-4-4) matched those of the strains from Şırnak, Iğdır, Ağrı, and Bingöl provinces in Turkey (Kiliç et al., 2011). Meanwhile, the profile of 2021/25-2 (Mersin) (1-5-3-13-3-2-3-2-4-41-8-5-4-3-3-7) did not exactly match any isolate in the database due to a variant in the Bruce-16 region. The MLVA8 (Panel-1) profile (1-5-3-13-3-2-3-2) of both strains was determined to be genotype 43. MLVA-11 (Panel-1,2A) profiles were detected as genotype 125. Furthermore, 2018/63-8 and 2021/25-2 were determined to be belonging to ST8 (3-2-3-2-1-5-3-2-8) based on MLST-9 typing (Table 2).

This study is the first report of *B. melitensis* bv2 infection in cattle in Turkey. The detection of *Brucella* species and subspecies is critical in determining pathogen prevalence, disease epidemiology, and infection risk, as well as in determining prevention strategies (De Massis et al., 2019). *B. melitensis* is the most frequently isolated species in many countries of the Middle East (Rossetti et al., 2017), and *B. meliten-*

Table 1: Biotyping results of the two *Brucella* strains recovered from cattle and reference strains.

	Sample information				***Brucella* spp. growth characteristics**													**Results**
	Strain NO	Host	Province	District	Urease	CO_2 requirement	H_2S production	Growth on dyes			Growth on antibiotic			Lysis by phage		Agglutination with monospecific antisera			
								Thionine	Fuchsin	Safranine	Penicillin	Streptomycin	İ-erythritol	Tibilis	R/C	A	M	R	
1	2018/83-63 (11)	Cattle	Erzurum	Olur	+	-	-	+	+	+	+	-	+	-	-	+	-	-	*B. melitensis* bv2
2	2021/25-2 (12)	Cattle	Mersin	Tarsus	+	-	-	+	+	+	+	-	+	-	-	+	-	-	*B. melitensis* bv2
3	Reference *B. melitensis* Rev-1 strain				+	-	-	-	-	-	-	+	+	-	-	-	+	-	*B. melitensis* Rev-1
4	Reference *B. melitensis* bv1 (16M)				+	-	-	+	+	+	+	-	+	-	-	-	+	-	*B. melitensis* bv1
5	Reference *B. melitensis* bv2 (63/9)				+	-	-	+	+	+	+	-	+	-	-	+	-	-	*B. melitensis* bv2
6	Reference *B. melitensis* bv3 (Ether)				+	-	-	+	+	+	+	-	+	-	-	+	+	-	*B. melitensis* bv3
7	Reference B. abortus S-19 vaccine strain				-	-	-	+	-	-	-	-	+	-	+	-	-	-	*B.abortus* S-19
8	Reference B.abortus bv1(544)				+	+	-	+	+	+	-	+	+	-	+	-	+	+	*B.abortus* bv1
9	Reference B.abortus bv2(86/8/59)				+	+	-	-	-	-	-	+	+	-	+	-	+	+	*B.abortus* bv2
10	Reference B.abortus bv3(Tulya)				+	+	+	+	+	+	-	+	+	-		-	+	+	*B.abortus* bv3
11	Reference B.abortus bv4(292)				+	+	-	+	+	+	-	+	+	-	-	+	+	+	*B.abortus* bv4
12	Reference B.abortus bv9(C68)				+	+	+	+	+	+	-	+	+	-	-	+	+	+	*B.abortus* bv9

Table 2: Previous studies and Multilocus Sequence Typing values of isolates from Turkey in the database (pubmlst.org) (Akar and Erganis, 2022). Strain 2018/83-63 and 2021/25-2 are the two strains investigated in the current study, ST refers to MLST type.

Strains	Host	Biovar	Year	*gap*	*aroA*	*glk*	*dnaK*	*gyrB*	*trpE*	*cobQ*	*int_hyp*	*omp25*	ST
Pubmlst.org	Unknown	3	2005	3	2	3	2	1	5	3	2	8	8
Pubmlst.org	Ovine	3	1965	3	2	3	2	1	5	3	2	8	8
Pubmlst.org	Human	2	2001	3	2	3	2	1	5	3	2	8	8
Pubmlst.org	Human	3	2007	3	2	3	2	1	5	3	2	8	8
Pubmlst.org	Human	3	2011	3	2	3	2	1	5	3	2	8	8
Pubmlst.org	Human	-	2007	3	2	3	2	1	5	3	2	8	8
Pubmlst.org	Human	-	2014	3	2	3	2	1	5	3	2	8	8
Akar and Erganis, 2022	Goat	1	2017	3	2	35	2	1	5	3	2	8	102
Akar and Erganis, 2022	Sheep	1	2017	3	2	35	2	1	5	3	2	8	102
Akar and Erganis, 2022	Cattle	3	2012	3	2	35	2	1	5	3	2	8	102
Akar and Erganis, 2022	Sheep	2	2009	3	2	3	2	1	5	3	2	8	8
Akar and Erganis, 2022	Sheep	2	2013	3	2	3	2	1	5	3	2	8	8
Akar and Erganis, 2022	Cattle	1	2012	3	2	3	2	1	5	3	2	8	8
Akar and Erganis, 2022	Cattle	3	2012	3	2	3	2	1	5	3	2	8	8
2018/83-63	Cattle	2	2018	3	2	3	2	1	5	3	2	8	8
2021/25-2	Cattle	2	2021	3	2	3	2	1	5	3	2	8	8

sis bv2 has previously been isolated from sheep and goats in Turkey (Karagul et al., 2017). Cases of abortion in cattle caused by *B. melitensis* have been reported in many countries, including Spain, Italy, Syria, Egypt, and Oman (Darwish and Benkirane, 2001; Alvarez et al., 2011; Foster et al., 2017; El-Diasty et al., 2018; De Massis et al., 2019). Furthermore, *B. melitensis* bv2 has been reported in cattle in Algeria (Lounes et al., 2021).

According to MLVA-8 and MLVA-11, these findings are similar to the dominant genotype profiles obtained in previous Turkish studies (Kiliç et al., 2011; Akar et al., 2022). Furthermore, the ST8 dominant sequence type for Turkey was detected in both isolates. The MLVA-16 genotype of the 2018/63-8 strain matched with human isolates from Turkey in the MLVA database, indicating the importance of inter-host transmission and One-health. On the other hand, the 2021/25-2 strain genotype matched with Turkish isolates in the MLVA database, with variation only in the Bruce-16 locus (Kiliç et al., 2011). Further evaluation using whole genomic sequencing (WGS) and Proteomics is required for a better understanding of the interaction between the host and the pathogen.

Conclusion

Although *B. melitensis* bv3 is the most common species in Turkey, other *B. melitensis* biovars have been described. *B. melitensis* bv2 isolates from sheep and goats are limited. This study is the first report of *B. melitensis* bv2 isolation from cattle in Turkey. This finding suggests its presence in other species, although it has not yet been identified. It is seen as important epidemiological data in terms of the host diversity of *Brucella* spp. circulating in the field. Thus, this study may contribute to *Brucella*'s control strategy, an important zoonotic pathogen.

Article Information

Funding. The work was partially funded by ICRAD (BruceGenoProt) project 325-06.01-2821ERA27D as part of funding international research collaborations on world nutrition and other international research tasks in nutrition, agriculture, and consumer health protection.

Conflict of Interest. The authors declare no conflict of interest.

Acknowledgments. The authors thank Dr. Gamal Wareth for his support. Erzurum Veterinary Control Institute and Adana Veterinary Control Institute are acknowledged for their efforts in isolating these two strains.

Authors Contributions. AK and GYÖ contributed to the design, conception, data gathering, analysis, and interpretation. Authors drafted or edited the manuscript. The authors gave final approval of the published version.

References

Akar, K., Erganis, O., 2022. Evaluation of the genetic profiles of *Brucella melitensis* strain from Turkey using multilocus variable number tandem repeat analysis (MLVA) and multilocus sequence typing (MLST) techniques. Veterinary Microbiology 269, 109423. 10.1016/j.vetmic.2022.109423.

Akar, K., Tatar, F., Schmoock, G., Wareth, G., Neubauer, H., Erganiş, O., 2022. Tracking the diversity and mediterranean lineage of *Brucella melitensis* isolates from different animal species in Turkey using MLVA-16 genotyping. German Journal of Veterinary Research 2, 25–30. 10.51585/gjvr.2022.1.0037.

Alton, G., Jones, L., Angus, R., Verger, J., 1988. Techniques for the Brucellosis Laboratory (Techniques et Pratiques). Institute National de la Recherche Agronomique.

Alvarez, J., Sáez, J.L., García, N., Serrat, C., Pérez-Sancho, M., González, S., Ortega, M.J., Gou, J., Carbajo, L., Garrido, F., Goyache, J., Domínguez, L., 2011. Management of an outbreak of brucellosis due to *B. melitensis* in dairy

cattle in Spain. Research in Veterinary Science 90, 208–211. 10.1016/j.rvsc.2010.05.028.

Darwish, M., Benkirane, A., 2001. Field investigations of brucellosis in cattle and small ruminants in Syria, 1990-1996. Revue Scientifique et Technique (International Office of Epizootics) 20, 769–775. 10.20506/rst.20.3.1313.

De Massis, F., Zilli, K., Di Donato, G., Nuvoloni, R., Pelini, S., Sacchini, L., D'Alterio, N., Di Giannatale, E., 2019. Distribution of *Brucella* field strains isolated from livestock, wildlife populations, and humans in Italy from 2007 to 2015. Plos One 14, e0213689. 10.1371/journal.pone.0213689.

El-Diasty, M., Wareth, G., Melzer, F., Mustafa, S., Sprague, L., Neubauer, H., 2018. Isolation of *Brucella abortus* and *Brucella melitensis* from seronegative cows is a serious impediment in brucellosis control. Veterinary Sciences 5, 28. 10.3390/vetsci5010028.

Foster, J.T., Walker, F.M., Rannals, B.D., Hussain, M.H., Drees, K.P., Tiller, R.V., Hoffmaster, A.R., Al-Rawahi, A., Keim, P., Saqib, M., 2017. African lineage *Brucella melitensis* isolates from Omani livestock. Frontiers in Microbiology 8, 2702. 10.3389/fmicb.2017.02702.

Garofolo, G., Ancora, M., Di Giannatale, E., 2013. MLVA-16 loci panel on *Brucella* spp. using multiplex PCR and multicolor capillary electrophoresis. Journal of Microbiological Methods 92, 103–107. 10.1016/j.mimet.2012.11.007.

Jamil, T., Akar, K., Erdenlig, S., Murugaiyan, J., Sandalakis, V., Boukouvala, E., Psaroulaki, A., Melzer, F., Neubauer, H., Wareth, G., 2022. Spatio-temporal distribution of brucellosis in European terrestrial and marine wildlife species and its regional implications. Microorganisms 10. 10.3390/microorganisms10101970.

Karagul, M.S., Baklan, E.A., Saytekin, A.M., Altuntas, B., Oz, G.Y., Gurbilek, S.E., 2017. Biovar distribution of *Brucella* strains isolated from livestock in *Turkey* between 2010-2015. International Journal of Academic Research 10.18805/ijar.v0iOF.9180.

Kiliç, S., Ivanov, I.N., Durmaz, R., Bayraktar, M.R., Ayasli-oglu, E., Uyanik, M.H., Aliskan, H., Yasar, E., Bayramoglu, G., Arslantürk, A., Vergnaud, G., Kantardjiev, T.V., 2011. Multiple-locus variable-number tandem-repeat analysis genotyping of human *Brucella* isolates from Turkey. Journal of Clinical Microbiology 49, 3276–3283. 10.1128/JCM.02538-10.

Le Flèche, P., Jacques, I., Grayon, M., Al Dahouk, S., Bouchon, P., Denoeud, F., Nöckler, K., Neubauer, H., Guilloteau, L.A., Vergnaud, G., 2006. Evaluation and selection of tandem repeat loci for a *Brucella* MLVA typing assay. BMC Microbiology 6, 9. 10.1186/1471-2180-6-9.

Lounes, N., Melzer, F., Sayour, A.E., Maamar, H.T., Rahal, K., Benamrouche, N., Lazri, M., Bouyoucef, A., Hendam, A., Neubauer, H., El-Adawy, H., 2021. Identification, geographic distribution and risk factors of *Brucella abortus* and *Brucella melitensis* infection in cattle in Algeria. Veterinary Microbiology 254, 109004. 10.1016/j.vetmic.2021.109004.

Mayer-Scholl, A., Draeger, A., Göllner, C., Scholz, H.C., Nöckler, K., 2010. Advancement of a multiplex PCR for the differentiation of all currently described *Brucella* species. Journal of Microbiological Methods 80, 112–114. 10.1016/j.mimet.2009.10.015.

Rossetti, C.A., Arenas-Gamboa, A.M., Maurizio, E., 2017. Caprine brucellosis: A historically neglected disease with significant impact on public health. PLoS Neglected Tropical Diseases 11, e0005692. 10.1371/journal.pntd.0005692.

Wareth, G., Abdeen, A., Ali, H., Bardenstein, S., Blasco, J.M., Cardoso, R., De Sá, M.I.C., Cvetnić, , Massis, F., El-Diasty, M., Krt, B., Ocepek, M., 2019. Brucellosis in the Mediterranean countries: History, prevalence, distribution, current situation and attempts at surveillance and control. The World Organisation for Animal Health. Technical series, Vol.12, 2019), Paris, France. URL: https://www.openagrar.de/receive/openagrar_mods_00048479.

Wareth, G., Dadar, M., Ali, H., Hamdy, M.E.R., Al-Talhy, A.M., Elkharsawi, A.R., Tawab, A.A.A.E., Neubauer, H., 2022. The perspective of antibiotic therapeutic challenges of brucellosis in the Middle East and North African countries: Current situation and therapeutic management. Transboundary and Emerging Diseases 69, e1253–e1268. 10.1111/tbed.14502.

Whatmore, A.M., Perrett, L.L., MacMillan, A.P., 2007. Characterisation of the genetic diversity of *Brucella* by multilocus sequencing. BMC Microbiology 7, 34. 10.1186/1471-2180-7-34.

Yumuk, Z., O'Callaghan, D., 2012. Brucellosis in Turkey- An overview. International Journal of Infectious Diseases 16, e228–35. 10.1016/j.ijid.2011.12.011.