Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
30 (4): 549-557, 2024
DOI: 10.9775/kvfd.2024.31980

Research Article

Distribution of Species and Biotypes of *Brucella* Isolates Obtained from Sheep and Cattle Abortions

Elif ÇELİK [1 (*)] Aliye GÜLMEZ SAĞLAM [1] Fatih BÜYÜK [1] Salih OTLU [1] Mitat ŞAHİN [1,2] Özgür ÇELEBİ [3] Mustafa Reha COŞKUN [1] Seda DURHAN [1] Eray BÜYÜK [1] Yaren ERSOY [1]

[1] Kafkas University, Faculty of Veterinary Medicine, Department of Microbiology, TR-36300 Kars - TÜRKİYE
[2] Faculty of Veterinary Medicine, Kyrgyz-Turkish Manas University, Chingiz Aitmatov Campus,Djal, Bishkek 720038, KYRGYZSTAN
[3] Kafkas University, Faculty of Medicine, Basic Medical Sciences, Department of Medical Microbiology, TR-36300 Kars - TÜRKİYE

(*) **Corresponding author:** Elif ÇELİK
Cellular phone: +90 474 242 6836/5135
Cellular phone: +90 541 205 5281
Fax: +90 474 242 6853
E-mail: elif.celik@kafkas.edu.tr

How to cite this article?

Çelik E, Gülmez Sağlam A, Büyük F, Otlu S, Şahin M, Çelebi Ö, Coşkun MR, Durhan S, Büyük E, Ersoy Y: Distribution of species and biotypes of Brucella isolates obtained from sheep and cattle abortions.. *Kafkas Univ Vet Fak Derg,* 30 (4): 549-557, 2024.
DOI: 10.9775/kvfd.2024.31980

Article ID: KVFD-2024-31980
Received: 11.03.2024
Accepted: 17.05.2024
Published Online: 28.05.2024

Abstract

This study was carried out to evaluate *Brucella* spp. isolated from various tissue samples of aborted sheep and bovine fetuses sent to the laboratory of Department of Microbiology, Faculty of Veterinary Medicine, Kafkas University between 2011 and 2023 years and determine the *Brucella* species and biotype diversity that carry a higher risk for abortion complications in these animals. In this context, 155 *Brucella* spp. isolates obtained from aborted fetuses were identified by species-specific Bruce-ladder PCR and biotyped using conventional biotyping methods. As a result of the study, *B. melitensis* and *B. abortus* were identified in 92.5% (n=74) and 7.5% (n=6) of sheep, *B. abortus* and *B. melitensis* were identified in 80% (n=60) and 20% (n=15) of cattle, respectively. *B. melitensis* biotype 2 in sheep and *B. abortus* biotype 3 in cattle were found as the dominant biotypes in these definitive hosts. In the Kars region, where brucellosis is endemic, while the biotype responsible for cattle brucellosis (*B. abortus* biotype 3) maintained its dominance over a 20-year period, there is a profile change from *B. melitensis* biotype 3 to *B. melitensis* biotype 2 in sheep. Considering the period covered by the study and the sample size analyzed, the data obtained provide up-to-date and important information about *Brucella* species and biotypes in Kars region and the animal species that host these agents.

Keywords: Sheep, Cattle, *Brucella melitensis*, *Brucella abortus*, Biotype, Bruce-ladder multiplex PCR

Introduction

Brucellosis is a prevalent zoonotic bacterial disease worldwide, affecting a wide range of mammals, including humans [1]. In animals, brucellosis manifests with various clinical presentations such as abortion, infertility, retained placenta, orchitis, epididymitis and, rarely, arthritis [2]. This often results in severe economic losses, including reduced reproductive productivity of livestock and animal replacement costs associated with removal from the herd [3]. Human infections are associated with direct contact with infected animals or their products and ingestion of contaminated dairy products, particularly cheese and unpasteurized milk, and exposure to infectious aerosols. Human brucellosis is rarely fatal, but it significantly affects various body systems (reproductive, musculoskeletal, central nervous, etc.) and causes severe and sometimes permanent sequelae, including disability [4].

The etiological agent of brucellosis is a non-motile, non-capsulated, non-spore-forming, facultative intracellular Gram-negative coccobacilli [5]. To date, in the *Brucella* genus there are 12 closely related species have been described based on both genetic and immunological characteristics [6,7]. Members of the *Brucella* genus have strict host preferences, but recent adaptations of the classical *Brucella* species to new hosts have been remarkable. Among these, *Brucella abortus* and *Brucella melitensis* are included in the classical species by primarily effecting cattle and sheep/goats, respectively [2,7-9]. In modern *Brucella* systematics, *B. abortus* and *B. melitensis* are divided into some biotypes. *B. melitensis* has three biotypes (biotype 1-3) and *B. abortus* has 7 biotypes (biotype 1-6 and 9) [5]. Although both *Brucella* species have a preferred host, they can cause infection in more than one host through their different biotypes [10]. The characterisation and biotyping of *Brucella* species are

crucial at various stages such as molecular epidemiological studies, determining the geographical origin of the source of infection and determining the relationships between isolates [11], development of control and eradication policies following epidemiological studies and even monitoring and controlling the efficacy of vaccine strains [10]. Periodic monitoring of species and biotype distribution and taking an inventory every ten years would make it more feasible to implement in the areas mentioned above.

With the advances in molecular techniques and a better understanding of the genomes of various *Brucella* species, molecular methods based on PCR are widely used in both differential diagnosis and molecular epidemiologic studies [12]. In this context, multiplex PCR techniques such as Bruce-Ladder [13], have been developed that can identify *Brucella* isolates down to strain level and distinguish field strains from vaccine strains. However, the same principled methodology is still unable to distinguish *Brucella* biotypes. Biotyping of *Brucella* strains is still based on some characteristics of the isolates such as CO_2 requirement, H_2S production, urea hydrolysis, basic fuchsin and thionine sensitivity, and phage susceptibility, as well as agglutination with monospecific sera, and lysis by *Brucella* phages [14,15].

This study aimed to determine the *Brucella* species and biotypes distribution in clinical brucellosis cases in farm animals.

Material and Methods

Ethical Statement

This study vas approved by the Kafkas University Animal Experiments Local Ethics Committee (Approval no: KAÜ-HADYEK/2020/103).

Reference *Brucella* Strains

In the study, *B. abortus* 544, *B. melitensis* 16 M, *B. abortus* S19, *B. melitensis* Rev 1, *B. ovis* 63/290 strains belong to the culture collection kept at Department of Microbiology, Faculty of Veterinary Medicine of Kafkas University, Türkiye were used as reference *Brucella* strains.

Brucella Field Isolates

The study material was consisted of 155 *Brucella* isolates obtained from aborted sheep and cattle fetuses. *Brucella* isolates were obtained from samples such as lung, liver and abomasum contents of aborted fetuses sent to Kafkas University, Faculty of Veterinary Medicine, Department of Microbiology between 2011 and 2023.

Tissue and organ samples of aborted fetuses were cultured on blood agar containing 5-10% sheep blood. Double cultivation was performed for all samples and incubations were performed in aerobic and 5-10% CO_2 environment at 37°C for 5-7 days.

Colony morphology, Gram staining and growth characteristics, catalase, oxidase and urease test results were considered to identify the isolates in *Brucella* genus level [2]. Isolates previously isolated and identified as *Brucella* spp. were stored in *Brucella* Broth with 20% glycerin and stored at -80°C until the molecular identification and biotyping tests performed.

Species-Specific Identification of *Brucella* Isolates by PCR

- *Genomic DNA Extraction*

For the chromosomal DNA extraction, the single cell lysis buffer (SCLB) method was performed from fresh cultures of the *Brucella* isolates [16].

- *Bruce-Ladder Multiplex PCR*

Species-specific identification of the *Brucella* isolates was performed with Bruce-ladder PCR, a multiplex PCR [13]. The primers and target genes used in the Bruce-Ladder PCR are given in *Table 1*. Bruce-ladder multiplex PCR was consisted of 5 µL 5xLongAmp™ Taq Reaction Buffer, 1 µL of $MgCl_2$ (20 mM), 0.75 µL of dNTP (10 mM), 1 µL of each primer (12 pieces) (20 pmol), 1 µL of LongAmpR Taq DNA Polymerase (5 U) and 3 µL of template DNA (50 ng/µL). The thermal condition of Bruce-ladder PCR was set with an initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 35 sec, primer binding at 62°C for 45 sec, elongation at 65°C for 3 min and a final elongation at 65°C for 10 min. Amplified products were analysed on 1.5% agarose gel. The band sizes obtained were evaluated and *Brucella* isolates were identified at species-level considering the band combination in *Table 1*.

Biotyping of *Brucella* Isolates

Biotyping of the *Brucella* isolates were performed based on H_2S production, growth in the presence of thionine and basic fuchsin dyes, and agglutination reactions with mono-specific A and M anti-sera [14,15,17]. Tryptic soy agar (TSA) (Merck, 105458) supplemented with dextrose and heat inactivated horse serum solution (5%) and was employed as the basal medium for all cultural examinations. Inoculated these plates were incubated at 37°C in condiditions with 5-10% CO_2 for 4-5 days [9].

- *CO_2 Requirement and H_2S Production*

CO_2 requirement for growth was evaluated in first isolation of strains. For H_2S production test, paper strips with lead acetate were placed between the tube edge and the screw cap so that they did not come into contact with the TSA medium. For each *Brucella* isolate, inoculation was incubated in 5-10% CO_2 at 37°C for 4-5 days. At the end of the incubation period, the results were evaluated according to the color change in the lead acetate papers [14,15].

Table 1. *List of primer pairs used for Bruce-ladder multiplex PCR and the reaction results of the Brucella species*

Primer	Sequence (5'–3')	Amplicon size (bp)	DNA targets	Strains
BMEI0998f	ATCCTATTGCCCCGATAAGG	1682	Glycosyltransferase, gene *wboA*	*B. abortus* *B. melitensis* *B. suis* *B. canis* *B. abortus* S19 *B. melitensis* Rev 1
BMEI0997r	GCTTCGCATTTTCACTGTAGC			
BMEII0843f	TTTACACAGGCAATCCAGCA	1071	Outer membrane protein, gene *omp31*	*B. melitensis* *B. ovis* *B. suis* *B. canis* *B. melitensis* Rev 1
BMEII0844r	GCGTCCAGTTGTTGTTGATG			
BMEI1436f	ACGCAGACGACCTTCGGTAT	794	Polysaccharide deacetylase	*B. abortus* *B. melitensis* *B. ovis* *B. suis* *B. abortus* S19 *B. melitensis* Rev 1
BMEI1435r	TTTATCCATCGCCCTGTCAC			
BMEII0428f	GCCGCTATTATGTGGACTGG	587	Erythritol catabolism, gene *eryC* (D-erythrulose-1-phosphate dehydrogenase)	*B. abortus* *B. melitensis* *B. ovis* *B. suis* *B. canis* *B. melitensis* Rev 1
BMEII0428r	AATGACTTCACGGTCGTTCG			
BMEI0535f	GCGCATTCTTCGGTTATGAA	450	Immunodominant antigen, gene *bp26*	*B. abortus* *B. melitensis* *B. ovis* *B. suis* *B. canis* *B. abortus* S19 *B. melitensis* Rev 1
BMEI0535r	CGCAGGCGAAAACAGCTATAA			
BR0953f	GGAACACTACGCCACCTTGT	272	ABC transporter binding protein	*B. suis* *B. canis*
BR0953r	GATGGAGCAAACGCTGAAG			
BMEI0752f	CAGGCAAACCCTCAGAAGC	218	Ribosomal protein S12, gene *rpsL*	*B. melitensis* Rev 1
BMEI0752r	GATGTGGTAACGCACACCAA			
BMEII0987f	CGCAGACAGTGACCATCAAA	152	Transcriptional regulator, CRP family	*B. abortus* *B. melitensis* *B. ovis* *B. suis* *B. canis* *B. abortus* S19 *B. melitensis* Rev 1
BMEII0987r	GTATTCAGCCCCCGTTACCT			

- Growth in the Presence of Thionine and Basic Fuchsin Dyes

Brucella colonies cultivated freshly on TSA supplemented with dextrose and heat inactivated horse serum solution were collected with peptone-saline from the agar surface, and a bacterial inoculum was prepared containing approximately 1x10^9 CFU/mL bacteria via McFarland standard 4.

Inoculations were done with a steril swab from suspansion of field isolates and standard strains onto TSA slides containing thionine and basic fuchsin (20 µg/mL) As the expression is the same, it would be sufficient to use only one of them. Slides were incubated in at 37°C 5-10% CO_2 for 4-5 days. The results were evaluated according to their growth status [10,14].

- Agglutination with Mono-Specific Anti-Sera (A and M)

A loopful of bacterial colony was taken from the fresh culture of each isolates to be evaluated and a bacterial suspension was prepared in 0.25 mL of physiological saline. One drop of each monospecific antisera A and M were placed on a clean slide and one drop of bacterial suspension was added and mixed throughly. The results were analysed according to the agglutination within one minute [14,15].

Statistical Analysis

The Pearson Chi Square test, one of the nonparametric tests, was used to measure changes in the isolation rates of the obtained *B. abortus* and *B. melitensis* biotypes according to years and animal groups.

Results

Bruce-Ladder Multiplex PCR Results

As a result of the Bruce-ladder PCR, 74 (92.5%) of the *Brucella* isolates from sheep originated were identified as *B. melitensis* and 6 (7.5%) as *B. abortus*, while 60 (80%) of the *Brucella* isolates from cattle originated were identified as *B. abortus* and 15 (20%) as *B. melitensis*. It was observed that all of the *Brucella* isolates identified were field strains.

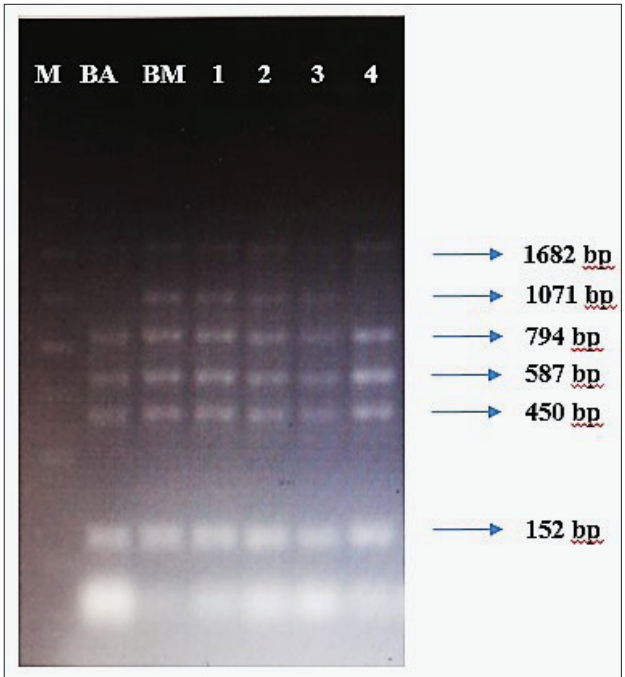

Fig 1. 1.5% agarose gel electrophoresis images of Bruce-ladder PCR products. M: GeneRuler 1 kb DNA Ladder (Thermo Sci SM0311). BA: *B. abortus* biotype 1 (544), BM: *B. melitensis* biotype 1 (16M), 1-3: Field strain (*B. melitensis*), 4: Field strain *(B. abortus)*

Fig. 1 shows the agarose gel electrophoresis analysis of the amplified products of the *Brucella* strains identified by Bruce-ladder multiplex PCR.

Biotyping Results

In the present study, a total of 155 *Brucella* isolates (80 from sheep and 75 from cattle) were biotyped using conventional methods. Out of the 74 (92.5%) *B. melitensis* isolates obtained from sheep, 43 (58.1%) were biotyped as *B. melitensis* biotype 2, 21 (28.4%) as *B. melitensis* biotype 3 and 10 (13.5%) as *B. melitensis* biotype 1. Among the 6 *B. abortus* isolates obtained from sheep, 3 (50%) were biotyped as *B. abortus* biotype 1 and 3 (50%) as *B. abortus* biotype 3 *(Table 2)*.

Out of the 60 *B. abortus* isolates obtained from cattle, 29 (48.33%) were biotyped as *B. abortus* biotype 3, 23 (38.33%) as *B. abortus* biotype 1, and 8 (13.33%) as *B. abortus* biotype 2. Among the 15 cattle *B. melitensis* isolates, 10 (67%) were biotyped as *B. melitensis* biotype 3, 3 (20%) as *B. melitensis* biotype 2, and 2 (13%) as *B. melitensis* biotype 1 *(Table 2)*.

In Kars province, given the isolation rates of biotypes according to year, it is seen that among the sheep-originated strains, *B. melitensis* biotype 3 was predominant in 2004-2006 *(Table 3)*, 2015, 2019, 2021, and 2023 *(Table 4)*, and *B. melitensis* biotype 1 in 2011, 2014, and 2016 *(Table 4)*. Interestingly *B. melitensis* biotype 2 was dominant in 2012, 2017, 2018, 2020 and 2022 in sheep *(Table 4)*. And all these *B. melitensis* biotypes were found in 2013 and 2016 *(Table 4)*. When analyzing the strain distributions in five-year periods, it is seen that *B. melitensis* biotype 2 maintained its presence between 2011-2015 and 2016-2020, and *B. melitensis* biotype 2 and biotype 3 maintained their presence between 2021-2023 *(Table 4)*.

B. abortus biotype 3 was the dominant biotype in cattle in 1998-2010 *(Table 3)* and in 2011, 2012, 2016-2018 and 2022 *(Table 5)*, whereas, *B. abortus* biotype 1 was the dominant biotype in 1998-2002 *(Table 3)* and 2013, 2018, 2020-2023 *(Table 5)*. *B. abortus* biotype 2 was the only biotype isolated in 2019 *(Table 5)*. Therefore, as a result

Table 2. *Properties and distribution of B. melitensis and B. abortus biotypes*

Biotype	Characteristics						Origins		P Value
	CO_2^*	H_2S^{**}	BF	T	A	M	Sheep (%)	Cattle (%)	
B. melitensis biotype 1	-	-	+	+	-	+	10 (13.5)	2 (13)	0.022
B. melitensis biotype 2	-	-	+	+	+	-	43 (58.1)	3 (20)	0.000
B. melitensis biotype 3	-	-	+	+	+	+	21 (28.4)	10 (67)	0.000
B. abortus biotype 1	+	+	+	-	+	-	3 (50)	23 (38.33)	0.000
B. abortus biotype 2	+	+	-	-	+	-	0 (0)	8 (13.3)	0.000
B. abortus biotype 3	+	+	+	+	+	-	3 (50)	29 (48.33)	0.000

** CO_2 requirement, ** H_2S Production, **BF:** Growth in Basic Fuchsin, **T:** Growth in Thionin, **A:** Aglutination with Antisera A, **M:** Aglutination with Antisera M*

Table 3. *Results obtained from biotyping studies for sheep-cattle brucellosis in Kars province*

Year	Origin	*B. melitensis* biotype 1 (n, %)	*B. melitensis* biotype 2 (n, %)	*B. melitensis* biotype 3 (n, %)	*B. abortus* biotype 1 (n, %)	*B. abortus* biotype 2 (n, %)	*B. abortus* biotype 3 (n, %)	Total Number of the Isolate	Reference
2004-2006	Sheep	0 (0)	0 (0)	25 (100)	0 (0)	0 (0)	0 (0)	25	[18]
2011-2023		10 (12.5)	43 (53.75)	21 (26.25)	3 (3.75)	0 (0)	3 (3.75)	80	Present study
1998-2002	Cattle	0 (0)	0 (0)	0 (0)	13 (46.43)	0 (0)	15 (53.57)	28	[19]
1998-2002		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	25 (100)	25	[20]
2001-2006		0 (0)	0 (0)	0 (0)	3 (6.25)	0 (0)	45 (93.75)	48	[21]
2008		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16 (100)	16	[22]
2009-2010		0 (0)	0 (0)	1 (3.2)	0 (0)	0 (0)	30 (96.8)	31	[23]
2008-2010		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	25 (100)	25	[24]
2011-2023		2 (2.7)	3 (4)	10 (13.3)	23 (30.7)	8 (10.6)	29 (38.7)	75	Present study

Table 4. *Distribution of identified biotypes in sheep in the study according to years*

Year	The Number of Isolates	Identified Biotypes					
		B. melitensis biotype 1	*B. melitensis* biotype 2	*B. melitensis* biotype 3	*B. abortus* biotype 1	*B. abortus* biotype 2	*B. abortus* biotype 3
2011	13	6	5	1	0	0	1
2012	11	1	9	1	0	0	0
2013	7	2	2	2	1	0	0
2014	4	0	0	1	2	0	1
2015	2	0	0	2	0	0	0
2016	3	1	1	1	0	0	0
2017	10	0	8	2	0	0	0
2018	1	0	1	0	0	0	0
2019	1	0	0	1	0	0	0
2020	13	0	10	3	0	0	0
2021	7	0	1	6	0	0	0
2022	7	0	6	0	0	0	1
2023	1	0	0	1	0	0	0

Table 5. *Distribution of identified biotypes in cattle in the study according to years*

Year	The Number of Isolates	Identified Biotypes					
		B. abortus biotype 1	*B. abortus* biotype 2	*B. abortus* biotype 3	*B. melitensis* biotype 1	*B. melitensis* biotype 2	*B. melitensis* biotype 3
2011	8	0	0	5	1	0	2
2012	7	2	0	5	0	0	0
2013	4	3	0	1	0	0	0
2015	4	0	0	0	0	1	3
2016	7	0	0	6	0	0	1
2017	5	0	0	5	0	0	0
2018	8	4	0	3	0	0	1
2019	8	1	4	1	0	1	1
2020	12	3	4	2	1	0	2
2021	7	6	0	0	0	1	0
2022	2	1	0	1	0	0	0
2023	3	3	0	0	0	0	0

of the Chi-Square analysis (χ^2 = 154.362; P=0.000), it was seen that there was a statistically significant difference in the diversity of biotype isolation according to years.

Discussion

Brucellosis is recognized as one of the global zoonoses that causes significant economic losses in the livestock industry worldwide and poses a serious public health problem [25]. Animal brucellosis is mainly characterized by reproductive system diseases [25], resulting in some clinical signs such as placental retention, birth of weak offspring, dead offspring, infertility and abortion [26]. In livestock, herd identification of brucellosis is usually based on the typical clinical signs, especially serological examinations and isolation of the causative agent. In any case, the diagnosis of brucellosis in one or more infected animals indicates the infection in a population. Therefore, detecting the causative agent in species or biotype based is extremely important in supporting the rapid and accurate diagnosis of Brucellosis and critical in the control and eradication of the infection in livestock [27].

Direct laboratory diagnostic methods of brucellosis in animals, such as bacterial isolation, have high specificity, but are time-consuming and require an appropriate degree of biosecurity. PCR-based molecular methods have been reported as an effective tool for rapid detection and confirmation of *Brucella* infection as well as for differentiation of *Brucella* species. Both culture and molecular techniques can definitively demonstrate the presence of infection and are effective in identifying the responsible *Brucella* species [28]. It has been emphasized that a molecular technique such as Bruce-ladder, which has been shown to work particularly well on *Brucella* spp. DNA and therefore will not be adversely affected by non-target DNA in contaminated agents, is a powerful technique both in the diagnosis of *Brucella* agents and in the differentiation of field and vaccine strains [9]. So, in the present study, Bruce-ladder multiplex PCR technique which has the discrimination power including vaccine strains, was applied to confirm the identification of the isolates obtained at the species level. The isolates obtained from sheep and cattle were identified as *B. abortus* and *B. melitensis*. All isolates were found to be field strains.

Brucella melitensis and *B. abortus* are predominant agents for small ruminants and cattle, respectively. Although a very strict host preference is observed in the *Brucella* species, the genus members have a wide host diversity [23]. These species are among the pathogenic bacteria that tend to adapt to new hosts and can be transmitted naturally to their primary hosts through direct or indirect ckontact and sometimes incidentally to other susceptible hosts [29]. While cross-infections between species were once rare, they have now become almost commonplace [12]. This situation has been reported in many studies. Abortions associated with *B. abortus* in sheep [30-33] and *B. melitensis* in cattle [30,32,34] have been widely reported. This situation has often been interpreted as the possibility that animals may be more likely to be exposed to *Brucella* infection, especially those originating from different species of agents, due to the coexistence of different animal species [9,10,12,26]. However, it was emphasized that this situation may be accompanied by factors such as the infectivity of the pathogenic agent, the immune structure of the host and the structure of the animal population [9]. In the present study, 20% (15/75) of the cattle-originated *Brucella* strains were identified as *B. melitensis* and 7.5% (6/80) of the sheep-originated *Brucella* strains were identified as *B. abortus*. These results, as in the aforementioned studies, show that the dominant species circulating in livestock can overcome host species barriers and adapt to new hosts despite their known host preferences. This is also an indication that in the Kars region, where Brucellosis is frequently seen and livestock farming is intensive, cattle and sheep are usually kept together and it is inevitable that these animals are exposed to the different *Brucella* species and the emergence of the cross-infection is inevitable. Therefore, the identification of the species involved in animal brucellosis, their potential to adapt to new environments and the changes that may occur in their epidemiological characteristics can be considered as effective factors to be taken into consideration in the design of protection and control programs to solve the complexity of the interactions of these microorganisms with each other as well as their interactions with animals and humans.

It has been revealed that different biotypes are effective in bovine brucellosis in many countries of the World. In Egypt [1], Tanzania [6], Italy [8], Yemen [17], Bangladesh [31], Iran [32], South Africa [34], many West African countries [35], *B. abortus* biotype 3 was reported as the dominant biotype in cattle, whereas, *B. abortus* biotype 1 was dominant in some Latin American countries [36], Zimbabwe [37] and Brazil [38]. *B. abortus* biotype 3 is the dominant biotype in bovine brucellosis in Türkiye, as well. Sarısayın et al.[39] identified 87.93%, 7.76% and 0.86% of bovine *Brucella* isolates as *B. abortus* biotype 3, *B. abortus* biotype 1 and *B. abortus* biotype 2, respectively. Şahin et al.[21] identified 93.75% and 6.25% of which originated aborted bovine foetus isolates as *B. abortus* biotype 3 and biyotype 1, respectively. Büyükcangaz and Şen [40] identified *B. abortus* biotype 3 from bovine aborted fetuses at a rate of 87.5%. Büyük and Şahin [23] reported that the dominant biotype was *B. abortus* biotype 3 in milk and aborted bovine fetus samples. Gürbilek et al.[10] identified 96% of the isolates originated bovine as *B. abortus* biotype 3 in their studies on biotyping of 114 *Brucella* isolates different originated.

Erdenliğ Gürbilek et al.[9] identified 94.2% of the *Brucella* spp. isolated from cattle in 2009-2011 as *B. abortus* biotype 3. All isolates recovered from bovine originated samples were identified as *B. abortus* biotype 3 in other studies [31,41-44]. In the present study, it was observed that *B. abortus* biotype 3 was the predominant biotype in bovine *Brucella* isolates in accordance with the aforementioned studies. This shows that the causative species and biotype have not changed in bovine abortions and *B. abortus* biotype 3 is still the predominant in bovine brucellosis in our country and Kars province. However, Sözmen et al.[20] reported 81.8% and 18.2% of the isolates as *B. abortus* biotype 1 and *B. abortus* biotype 3, respectively. In the present study, 38.33% of *B. abortus* isolates were identified as *B. abortus* biotype 1. For this reason, it is thought to be important in terms of evaluating the agent as the second dominant species after *B. abortus* biotype 3. This situation also shows that the importance of species and biotype differences in the occurrence of the disease should be taken into consideration.

When sheep brucellosis is evaluated in various countries of the world, variability is observed between biotypes. Behroozikhah et al.[25], found 92.8% and 6.8% of the isolates as *B. melitensis* biotype 1 and *B. melitensis* biotype 2 in 2007-2009 in Iran. In Northern Cyprus, Demirpençe et al. [7], reported that the dominant biotype was *B. melitensis* biotype 1 followed by *B. melitensis* biotype 3. In addition, in Iran, Dadar and Alamian [45] reported that *B. melitensis* biotype 1 was dominant in sheep abortions, followed by *B. melitensis* biotype 2 and biotype 3 in 2016-2019. In Yemen, Al-Afifi et al.[17], isolated *B. melitensis* biotype 3, followed by *B. melitensis* biotype 2 in sheep. Various studies have been conducted on the biotype distribution of *B. melitensis* in Türkiye and *B. melitensis* biotype 3 was found the dominant biotype [9,10,30,42,44,46-48]. However, there are some studies reporting predominancy of *B. melitensis* biotype 1 and biotype 2 as well as *B. melitensis* biotype 3. Sarısayın et al.[39] identified 78% of sheep *Brucella* isolates as *B. melitensis* biotype 2 and 22% as *B. melitensis* biotype 1. In the present study, *B. melitensis* biotype 2 was the dominant biotype isolated from sheep abortions with a rate of 58.1%. However, this agent was followed by *B. melitensis* biotype 3 with 28.4% and *B. melitensis* biotype 1 with 13.5%. It is thought that this result may be due to the fact that the prevalence of *B. melitensis* biotype 2 started to increase and thus became dominant in the Kars region. In addition, factors such as the year intervals in which the studies were conducted, the difference in the origin of the isolates of the geographical regions, or the emergence of some variant or atypical strains may be effective in obtaining different results. Therefore, it is considered to be of great importance to periodically determine the *Brucella* species and biotypes causing brucellosis in our country. In addition, similar to the present study, Refai [49] reported that the dominant biotype isolated from sheep and goats was *B. melitensis* biotype 2 in a study covering a 10-year period in Saudi Arabia.

In a province-based evaluation of cattle-sheep brucellosis, the dominant biotypes obtained since the first biotyping studies conducted in the Kars region have been reported as *B. abortus* biotype 3 and followed by *B. abortus* biotype 1 in cattle, and *B. melitensis* biotype 3 in sheep. Indeed, Genç and Kamber [19], reported the isolates they detected in cattle in 1998-2000 as *B. abortus* biotype 3 and following *B. abortus* biotype 1. Sözmen et al.[20], identified all strains as *B. abortus* biotype 3 in 1998 and 2002. Şahin et al.[21] typed the isolates as *B. abortus* biotype 3 and following *B. abortus* biotype 1 in 2001-2006. Beytut et al.[41], isolated and identified *B. abortus* biotype 3 from cows with a history of abortion due to *B. abortus* in the winter season of 2002. Çelebi and Otlu [22] typed all of the isolates as *B. abortus* biotype 3 in 2008 and Büyük and Şahin [23], identified the isolates as *B. abortus* biotype 3 (96.77%), *B. abortus* biotype 6 (4.87%), *B. abortus* biotype 9 (3.22%), *B. abortus* biotype 1 (2.43%) in 2009-2010. Dağ et al.[24], bio-typed all isolates as *B. abortus* biotype 3 in 2008-2010. When looking at the studies covering a 14-year period, it is seen that there is a stable situation in dominancy of biotype profile which is primarily *B. abortus* biotype 3 followed by biotype 1. However, different biotypes such as *B. abortus* biotype 6 and biotype 9 have been scarcely identified as abortive agents. Biotyping studies on sheep brucellosis in the Kars region are very limited. Şahin et al.[18], biotyped all isolates as *B. melitensis* biotype 3 in a study conducted in 2004-2006. In the current study covering the years 2011-2023, out of the 60 *B. abortus* isolates obtained from cattle, 29 (48.33%) were biotyped as *B. abortus* biotype 3, 23 (38.33%) as *B. abortus* biotype 3, 23 (38.33%) as *B. abortus* biotype 1, and 8 (13.33%) as *B. abortus* biotype 2. This result shows that the agent responsible for clinical brucellosis in cattle has not changed and is still *B. abortus* biotype 3, followed by *B. abortus* biotype 1. In sheep out of the 74 (92.5%) *B. melitensis* isolates obtained from sheep, 43 (58.1%) were biotyped as *B. melitensis* biotype 2, 21 (28.4%) as *B. melitensis* biotype 3 and 10 (13.5%) as *B. melitensis* biotype 1. In sheep, unlike the aforementioned study, *B. melitensis* biotype 2 became dominant and its prevalence started to increase. This may be due to the fact that the species and biotypes are responsible for brucellosis between regions within a country and even between flocks. And, uncontrolled animal movements can be thought of as a contributing factor.

In conclusion in light of the findings of this study and previous studies in this direction, it is understood that the most common biotypes in our country continue to be *B. abortus* 3 followed by *B. abortus* biotype 1 in cattle, but

unlike sheep, the prevalence of *B. melitensis* biotype 2 has started to increase and become the dominant biotype. Considering the period covered by this study (13 years) and the sample size analyzed, the data obtained provide up-to-date and important general information on *Brucella* species and biotypes in the Kars region and the animal species prone to exposure with these agents.

Declarations

Availability of Data and Materials: The data and materials of this study are available from the corresponding author (E. Çelik).

Funding Support: This study was not supported by an institution.

Ethical Statement: This study vas approved by the Kafkas University Animal Experiments Local Ethics Committee (Approval no: KAÜ-HADYEK/2020/103).

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

Author Contributions: EÇ: Design of study; DNA extraction, PCR analysis and biotyping of isolates, writing – review & editing; AGS: Design of study, PCR analysis and biotyping of isolates; FB: Design of study, evaluation the results, review; SO: Design of study, evaluation the results, review; MŞ: Design of study, evaluation the results, review; ÖÇ: Design of study, evaluation the results; MRC: PCR analysis of isolates; SD: DNA extraction and biotyping of isolates; EB: DNA extraction of isolates; YE: DNA extraction and biotyping of isolates.

References

1. Wareth G, El-Diasty M, Melzer F, Schmoock G, Moustafa SA, El-Beskawy M, Khater DF, Hamdy MER, Zaki HM, Ferreira AC, Ekateriniadou LV, Boukouvala E, Abdel-Glil MY, Menshawy AMS, Sancho MP, Sakhria S, Pletz MW, Neubauer H: MLVA-16 genotyping of *Brucella abortus* and *Brucella melitensis* isolates from different animal species in Egypt: Geographical relatedness and the Mediterranean Lineage. *Pathogens,* 9:498, 2020. DOI: 10.3390/pathogens9060498

2. OIE - World Organisation for Animal Health: Brucellosis *(Brucella bortus, B. melitensis* and *B. suis)* (infection with *B. abortus, B. melitensis* and *B. suis*). **In,** OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 355-398, Paris, 2018.

3. WHO - World Health Organisation: Brucellosis in Humans and Animals. http://www.who.int/csr/resources/publications/deliberate/WHO_CDS_EPR_2006_7/en/2018; *Accessed:* 12.02.2024.

4. Franco MP, Mulder M, Gilman, RH, Smits HL: Human Brucellosis. *Lancet Infect Dis,* 7, 775-786, 2007. DOI: 10.1016/S1473-3099(07)70286-4

5. Kurmanov B, Zincke D, Wanwen Su, Hadfield TL, Aikimbayev A, Karibayev T, Berdikulov M, Orynbayev M, Nikolich MP, Blackburn JK: Assays for identification and differentiation of *Brucella* species: A review. *Microorganisms,* 10 (8): 1584, 2022. DOI: 10.3390/microorganisms10081584

6. Mathew C, Stokstad M, Johansen TB, Klevar S, Mdegela RH, Mwamengele G, Michel P, Escobar L, Fretin D, Godfroid J: First isolation, identification, phenotypic and genotypic characterization of *Brucella abortus* biovar 3 from dairy cattle in Tanzania. *BMC Vet Res,* 11:156, 2015. DOI: 10.1186/s12917-015-0476-8

7. Demirpençe M, Saytekin AM, Sareyyupoğlu B, Esendal ÖM: Isolation and characterisation of *Brucella melitensis* by bacteriological and molecular methods from livestock in North Cyprus. *Vet Med,* 67 (10): 497-509, 2022. DOI: 10.17221/150/2021-Vetmed

8. De Massis F, Zilli K, Di Donato G, Nuvoloni R, Pelini S, Sacchini L, D'Alterio N, Di Giannatale E: Distribution of *Brucella* field strains isolated from livestock, wildlife populations, and humans in Italy from 2007 to 2015. *PLoS One,* 14 (3):e0213689, 2019. DOI: 10.1371/journal.pone.0213689

9. Erdenliğ Gürbilek S, Baklan EA, Sağlam G, Karagül MS, Saytekin AM: Conventional and molecular identification of *Brucella* isolates from livestock in Turkey. *Ankara Univ Vet Fak Derg,* 69, 297-302, 2022. DOI: 10.33988/auvfd.796785

10. Erdenliğ Gürbilek S, Baklan EA, Aksoy HY: Identification and phage susceptibilities of *Brucella* strains in Turkey between the years 2007 and 2008. *Harran Üniv Vet Fak Derg,* 3 (2): 67-72, 2014.

11. Liu Z, Wang M, Zhao H, Piao D, Jiang H, Li Z: Investigation of the molecular characteristics of *Brucella* isolates from Guangxi province, China. *BMC Microbiol,* 19:292, 2019. DOI: 10.1186/s12866-019-1665-6

12. Çelik E, Kayman T, Büyük F, Gülmez Sağlam A, Abay S, Akar M, Karakaya E, Balkan Bozlak ÇE, Coşkun MR, Büyük E, Çelebi Ö, Şahin M, Satıcıoğlu İB, Durhan S, Baykal A, Ersoy Y, Otlu S, Aydın F: The canonical *Brucella* species-host dependency is changing, however, the antibiotic susceptibility profiles remain unchanged. *Microb Pathog,* 182:106261, 2023. DOI: 10.1016/j.micpath.2023.106261

13. Lopez-Goni I, Garcia-Yoldi D, Marin CM, de Miguel MJ, Munoz PM, Blasco JM, Jacques I, Grayon M, Cloeckaert A, Ferreira AC, Cardoso R, Correa de Sa MI, Walravens K, Albert D, Garin-Bastuji B: Evaluation of a multiplex PCR assay (Bruce-ladder) for molecular typing of all *Brucella* species, including the vaccine strains. *J Clin Microbiol,* 46 (10): 3484-3487, 2008. DOI: 10.1128/JCM.00837-08

14. Alton GG, Jones LM, Angus RD, Werger JM: Techniques for the Brucellosis Laboratory. INRA, Paris, France, 1988.

15. OIE - World Organisation for Animal Health: Brucellosis Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris: Office International des Epizooties, 2016.

16. Olah PA, Doetkott C, Fakhr MK, Logue CM: Prevalence of the *Campylobacter* multi-drug efflux pump (CmeABC) in *Campylobacter* spp. isolated from freshly processed turkeys. *Food Microbiol,* 23, 453-460, 2006. DOI: 10.1016/j.fm.2005.06.004

17. Al-Afifi A, Dhary AA, Al-Azazi ASH, Khalaf AM, Odhah MNA, Al-Gabri NA: Prevalence of *Brucella* spp. in milk from aborted and non-aborted animals in Dhamar governorate, Yemen. *Ital J Food Saf,* 11:10370, 2022. DOI: 10.4081/ijfs.2022.10370

18. Şahin M, Ünver A, Otlu S: Isolation and biotyping of *Brucella melitensis* from aborted sheep foetuses in Turkey. *Bull Vet Inst Pulawy,* 52, 59-62, 2008.

19. Genç O, Kamber U: Biotyping of *Brucella* strains isolated from abortions of cows in Kars province. *Ind Vet J,* 81, 1164-1165, 2004.

20. Sözmen M, Dağ Erginsoy S, Genç O, Beytut E, Özcan K: Immunohistochemical and microbiological detection of *Brucella abortus* in aborted bovine fetuses. *Acta Vet Brno,* 73, 465-472, 2004.

21. Şahin M, Genç O, Ünver A, Otlu S: Investigation of bovine brucellosis in the Northeastern Turkey. *Trop Anim Health Product,* 40, 281-286, 2008. DOI: 10.1007/s11250-007-9092-3

22. Çelebi O, Otlu S: Bacteriological and molecular description of *Brucella* species isolated from milk and vaginal swab samples of aborted cattle in Kars region. *Kafkas Univ Vet Fak Derg,* 17 (1): 53-58, 2011. DOI: 10.9775/kvfd.2010.2382

23. Büyük F, Şahin M: Invesigation of *Brucella* species from various samples of aborted cattle in Kars Province (Turkey) by cultural and molecular methods and epidemiological analysis of cases. *Kafkas Univ Vet Fak Derg,* 17 (5): 809-816, 2011. DOI: 10.9775/kvfd.2011.4509

24. Dağ S, Büyük F, Özen H, Çelebi Ö, Karaman M, Akça D, Şahin M: Detection of *Brucella* spp. in vaginal swab samples of aborting cattle: Comparison of immunoperoxidase to bacteriological culture technique. *Kafkas Univ Vet Fak Derg,* 18 (4): 617-622, 2012. DOI: 10.9775/kvfd.2012.6019

25. Behroozikhah AM, Bagheri NR, Amiri K, Bahonar, AR: Identification at biovar level of *Brucella* isolates causing abortion in small ruminants of Iran. *J Pathog,* 2012:357235, 2012. DOI: 10.1155/2012/357235

26. Dadar M, Alamian S, Behrozikhah AM, Yazdani F, Kalantari A, Etemadi A, Whatmore AM: Molecular identification of *Brucella* species and biovars associated with animal and human infection in Iran. *Vet Res Forum,* 10 (4): 315-321, 2019. DOI: 10.30466/vrf.2018.89680.2171

27. Saavedra MJ, Fernandes C, Queiroga C: Laboratory diagnosis of brucellosis. **In,** Simomes JCC, Saavedra MJ, Hunter PA (Eds): Brucellosis in Goats and Sheep: An Endemic and Re-emerging Old Zoonosis in the 21st Century. 151-180, New York, Nova Science Publishers, Inc, 2019.

28. Hosein HI, Zaki HM, Safwat NM, Menshawy AMS, Rouby S, Mahrous A, Madkour BE: Evaluation of the general organization of veterinary services control program of animal brucellosis in Egypt: An outbreak investigation of brucellosis in buffalo. *Vet World,* 11 (6): 748-757, 2018. DOI: 10.14202/vetworld.2018.748-757

29. Moreno E: Retrospective and prospective perspectives on zoonotic brusellosis. *Front Microbiol,* 13 (5):213, 2014. DOI: 10.3389/fmicb.2014.00213

30. Karagül MS, Baklan EA, Saytekin AM, Altuntas B, Öz GY, Gürbilek SE: Biovar distribution of *Brucella* strains isolated from livestock in Turkey between 2010-2015. *Indian J Anim Res,* 52, 1476-81, 2018.

31. Kolo FB, Adesiyun AA, Fasina FO, Katsande CT, Dogonyaro BB, Potts A, Matle I, Gelaw AK, van Heerden H: Seroprevalence and characterization of *Brucella* species in cattle slaughtered at Gauteng abattoirs, South Africa. *Vet Med Sci,* 5 (4): 545-55, 2019. DOI: 10.1002/vms3.190

32. Alamian S, Dadar M, Wareth G: Role of *Brucella abortus* biovar 3 in the outbreak of abortion in a dairy cattle herd immunized with *Brucella abortus* iriba vaccine. *Arch Razi Inst,* 75 (3): 377-384, 2020. DOI: 10.22092/ari.2019.125468.1305

33. Aslan S, Kandemir T, Uprak NS, Nagıyev T: The role of *Brucella abortus* strains in the abortion etiology of domestic ruminants in the Çukurova region, Turkey and molecular epidemiological characteristics. *Turk Hij Den Biyol Derg,* 80 (2): 201-212, 2023. DOI: 10.5505/TurkHijyen.2023.35033

34. Matle I, Ledwaba B, Madiba K, Makhado L, Jambwa K, Ntushelo N: Characterisation of *Brucella* species and biovars in South Africa between 2008 and 2018 using laboratory diagnostic data. *Vet Med Sci,* 7, 1245-1253, 2021. DOI: 10.1002/vms3.483

35. Sanogo M, Abatih E, Thys E, Fretin D, Berkvens D, Saegerman C: Importance of identification and typing of *Brucella* from West African cattle: A review. *Vet Microbiol,* 164, 202-211, 2013. DOI: 10.1016/j.vetmic.2013.02.009

36. Lucero NE, Ayala SM, Escobar GI, Jacob NR: *Brucella* isolated in humans and animals in Latin America from 1968 to 2006. *Epidemiol Infect,* 136, 496-503, 2008. DOI: 10.1017/S0950268807008795

37. Matope G, Bhebhe E, Muma JB, Skjerve E, Djønne B: Characterization of some *Brucella* species from Zimbabwe by biochemical profiling and AMOS-PCR. *BMC Res Notes,* 2 (1): 1-6, 2009. DOI: 10.1186/1756-0500-2-261

38. Minharro S, Mol JPS, Dorneles EMS, Pauletti RB, Neubauer H, Melzer F, Poester FP, Dasso MG, Pinheiro ES, Filho PMS, Santos RL, Heinemann MB, Lage AP: Biotyping and genotyping (MLVA16) of *Brucella abortus* isolated from cattle in Brazil, 1977 to 2008. *Plos One,* 8 (12):e81152, 2013. DOI: 10.1371/journal.pone.0081152

39. Sarısayın F, Eroğlu M, Nadas UG: Yurdumuzda izole edilen brusella suşlarının tür ve biyotiplerinin tayini ile dağılış durumu üzerine bir çalışma. *Pendik Vet Kont Araşt Derg,* 1, 24-35, 1969.

40. Büyükcangaz E, Şen A: The First isolation of *Brucella melitensis* from bovine aborted fetus in Turkey. *J Biol Environ Sci,* 1 (3): 139-142, 2007.

41. Beytut E, Şahin M, Erginsoy S, Sözmen M: Pathological, immunohistochemical, and bacteriological findings in the mammary glands and supramammary lymph nodes of cows with a history of abortion due to *Brucella abortus*. *Turk J Vet Anim Sci,* 33 (1): 37-43, 2009. DOI: 10.3906/vet-0705-12

42. İça T, Aydın F, Gümüşsoy KM, Percin D, Sümerkan AB, Ocak F, Abay S, Doğan HO, Fındık A, Çiftçi A: Conventional and molecular biotyping of *Brucella* strains isolated from cattle, sheep and human. *Ankara Üniv Vet Fak Derg,* 59, 259-264, 2012.

43. Erdenliğ Gürbilek S, Tel OY, Keskin O: Comparison of PCR and bacteriological culture to diagnose *Brucella* species in clinical samples collected from cows in brucellosis suspected herds. *Harran Üniv Vet Fak Derg,* 4 (2): 48-52, 2015.

44. Güven Gökmen T, Nagiyev T, Aslan S, Meral M, Etiz P, Yaman A, Köksal F: Molecular epidemiological characteristics of *Brucella* spp. isolated from human and animal brucellosis. *Indian J Anim Res,* 53, 399-403, 2019. DOI: 10.18805/ijar.B-978

45. Dadar M, Alamian S: Identification of main *Brucella* species implicated in ovine and caprine abortion cases by molecular and classical methods. *Arch Razi Inst,* 76 (1): 51-60, 2021. DOI: 10.22092/ari.2019.128003.1398

46. Erdenliğ S, Şen A: Koyun atıklarından izole edilen *Brucella* cinsi mikroorganizmaların izolasyonu ve biyotiplendirilmesi. *Pendik Vet Mikrobiol Derg,* 31 (2): 31-42, 2000.

47. Büyükcangaz E, Şen A, Kahya S: Isolation and biotyping of *Brucella melitensis* from aborted sheep and goat fetuses. *Turk J Vet Anim Sci,* 33 (4): 311-316, 2009. DOI: 10.3906/vet-0802-30

48. Büyükcangaz E, Şen A, Çarlı KT, Kahya S: Comparison of direct culture versus PCR for the detection of *Brucella* in aborted fetuses of cattle and sheep in Turkey. *Vet Rec,* 168:430, 2011. DOI: 10.1136/vr.c7003

49. Refai M: Incidence and control of brucellosis in the Near East region. *Vet Microbiol,* 90, 81-110, 2002. DOI: 10.1016/S0378-1135(02)00248-1