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A Genetic Comparison of *Brucella abortus* Isolates from Animals and Humans by Using the MLVA Assay

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The MLVA assay is known to have a high ability to identify and discriminate *Brucella* species, so that it can be used as an epidemiological tool to discriminate *Brucella* isolates originating from restricted geographic sources. In this study, the genetic profiles of 38 *B. abortus* isolates from humans were analyzed and compared with genotypes from animal isolates in South Korea. As a result, it was found that they did not show high genetic diversity and were compacted. They were clustered together with animal isolates, showing a significant correlation to regional distributions. With its ability to prove a significant genetic correlation among *B. abortus* isolates from animals and humans in South Korea, the MLVA assay could be utilized as part of a program to control and eradicate brucellosis, one of the major zoonoses. This study represents the first data of genetic correlation of *B. abortus* isolates from humans and animals in South Korea.

Keywords: *Brucella abortus*, MLVA, epidemiology, animal, human

The genus *Brucella* consists of six classical species, *B. ceti* (cetaceans) and *B. pinnipedialis* (seals) originating from marine mammals, and *B. microti* isolated from the common vole [9, 11, 14, 28, 34]. The *Brucella* species including *B. melitensis*, *B. abortus*, *B. suis*, *B. canis* and marine mammal strains are known to be pathogenic agents in humans [17, 27, 30, 36]. The *Brucella* species are transmitted to humans through a direct contact with the aborted fetuses, afterbirths, or uterine discharges of infected animals or with infected carcasses, or through a consumption of unpasteurized milk or cheese products, including raw milk [1, 11, 18]. Humans infected with the *Brucella* species usually develop symptoms similar to a severe influenza, accompanied particularly by undulant fever, which possibly progresses to a more chronic form or go so far as to produce serious complications affecting the muscular–skeletal, cardiovascular, and central nervous systems [1, 30].

In South Korea, bovine brucellosis was first detected among dairy cattle imported from the USA in 1955. Since then, the disease had occurred sporadically until 1983; most of its outbreaks were reported in dairy cattle [16, 20]. Notwithstanding the national eradication program employing the test-and-slaughter and/or stamp-out approach to strictly restrict and quarantine infected herds, brucellosis had continued to be increasingly prevalent among Korean native cattle called "Hanwoo" as well as dairy cattle. Specifically, there was a dramatic increase in 2004, which resulted from new introduced intensive regulations nationwide, including pre-marketing test certification systems to go so far as to cover Korean native cattle and beef cattle that had been neglected before. Since the year 2006 when it was at its peak (2.02%) in herd prevalence, bovine brucellosis has been on the gradual decrease, with 0.86% in prevalence in 2008 [15, 39]. Moreover, brucellosis in domestic elk was first reported in 2008 [12]. In fact, they might cause a significant threat to the public health through antler, deer blood, or any other products. Other *Brucella* species except for *B. abortus* and *B. canis* have not been reported over the last 20 years in South Korea [16].

In humans, one case of *B. abortus* infection was officially reported in 2002 [32]. Human infections have

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continued to rise nationwide, which resulted from enforcements or recommendations to test brucellosis among livestock farmers, employees, and any other epidemiological relatives engaging in the farms with reactors. Since the highest incidence of 215 cases in 2006, human brucellosis had decreased to 58 cases in 2008 [19].

Recently, the MLVA [multi-loci VNTR (variable-number tandem repeats) analysis] assay for the genus *Brucella* has been known to have a high ability to identify species and discriminate *Brucella* isolates originating from restricted geographic sources, indicating its potential to be used as an epidemiological tool [2, 4, 7, 10, 17, 22, 25–26, 35, 40]. With regard to other pathogenic bacterial species with a high genetic homogeneity, the MLVA assay has been proven to be a rapid and effective technique for assessing a discriminatory potential for genotype-based typing and epidemiological trace-back [23].

To effectively prevent and control brucellosis, one of the major zoonotic diseases in South Korea, there was an investigation to identify the genetic polymorphisms of *B. abortus* isolates originating from humans and their relatedness for regional distributions of animal isolates, by using the MLVA assay. On top of that, the MLVA assay was evaluated in terms of the possibility that it can be used as a molecular-based epidemiological tool to inhibit or reduce transmission of the diseases among animals as well as humans.

MATERIALS AND METHODS

Brucella Strains and DNA Preparation

DNAs from 38 *Brucella* isolates orginated from humans during the period ranging from 2003 to 2007 and any other related information were collected from KCDC (Table 1). The genomic DNA of the *Brucella* isolates was extracted using a DNeasy blood and tissue kit (Qiagen Korea Ltd., Korea), according to the manufacturer's instructions, and was stored at −20°C until further use. All isolates were reconfirmed as *B. abortus* by the use of the AMOS and Bruce-ladder PCR [5, 21].

MLVA Assay and Data Analysis

Seventeen loci for the MLVA typing assay consist of the primer sets of 16 loci and Hoof 3 as described by Al Dahouk *et al*. [2] and Bricker *et al*. [4, 22]. With regard to the primer sets of the 17 loci, their TRs copy number verification was performed in accordance with methods reported previously [13]. Clustering analysis was carried out by the use of UPGMA (unweighted pair group method using arithmetic averages) with categorical similarity coefficient, and the maximum parsimony was analyzed through a character dataset by the use of BioNumerics ver. 5.1 (Core-Bio, Korea).

Comparison of Genetic Profiles for *Brucella* Isolates

The genetic profiles of 38 *B. abortus* isolates from humans were compared with 23 genotypes of animals reported previously [13], which had originated from 177 *B. abortus* isolates in 105 cattle farms (including one elk farm) during the period 1996 to 2008 in South Korea. In addition, there was an investigation to identify the genetic co-relation with *B. abortus* isolates from animals and humans, and to analyze the characteristics of their regional distributions.

RESULTS

Thirty-eight *Brucella* isolates from humans were analyzed by the use of the MLVA assay using the 17 loci as described previously [2, 4, 22]. Diversity was seen at only 4 of the 17 loci (Bruce 43, Bruce 30, Hoof 3, and Bruce 04). Bruce 43 appeared to have the highest variability. In clustering analysis, human isolates were embedded into *B. abortus* strains and divided into 12 genotypes (Fig. 1). The most frequent genotypes, as compared with MLVA profiles of animal isolates [13], were D3, E4, and E1 (Table 1). The highest frequency genotypes distributed in humans were significantly similar to those in animal isolates. The D, E, and G clusters were distributed nationwide. The E4 genotype particularly appeared only at Gyeongbuk and its closest province, Daegu, during 2004–2006. The C5, E5, and I2 genotypes, however, were new types to be detected from humans (Table 1), as compared with the MLVA profiles of animal isolates [13]. They showed only one TR change (increase or decrease) in one to two loci, as compared with other genotypes.

The occupations of patients that originated the 38 *B. abortus* isolates, except for two veterinarians, were mainly livestock (cattle) farmers (Table 1). The genotypes of *B. abortus* isolates from two veterinarians were D3 and E1, which were popular types distributed nationwide. Twenty-six of 36 patients were livestock farmers managing cattle farm with serological reactors, and the remaining 10 patients were livestock workers or cohabited relatives of positive farms for bovine brucellosis, or livestock farmers near brucellosis outbreak farms.

Maximum parsimony tree analysis for *B. abortus* human isolates with a focus on evolutionary modeling was carried out, and compared with 23 genotypes of *B. abortus* animal isolates in South Korea [13]. Korean isolates from humans and animals were compactly clustered, and did not show high genetic diversity (Fig. 2). Two clusters (B and H), as shown in restricted provinces (north of Gyeonggi and Gyeongnam), were detected only in animals, whereas the remaining seven clusters were detected in both humans and animals.

DISCUSSION

Thirty-eight *Brucella* isolates from humans were analyzed using the 17 loci of the MLVA assay, and then compared with genetic profiles in animal isolates in South Korea. In

Table 1. Genetic profiles and the regional distribution of 38 *B. abortus* isolates from humans.

Species	Strain	Host	Occupation	Year	Province	MLVA profiles[a]	Genotype[b]	Comments
B. abortus	#2	Human	Livestock farmer	2003	Gangwon	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#3	Human	Veterinarian	2003	Gangwon	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#4	Human	Livestock farmer	2003	Jeonbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-2-3-3-5	F1	
B. abortus	#6	Human	Livestock farmer	2004	Chungbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#8	Human	Livestock farmer	2004	Gyeongnam	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#11	Human	Livestock farmer	2004	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-3	E4	
B. abortus	#12	Human	Livestock farmer	2004	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-3	E4	
B. abortus	#13	Human	Livestock farmer	2004	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-3	E4	
B. abortus	#17	Human	Livestock farmer	2004	Jeonbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#20	Human	Livestock farmer	2005	Jeonbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#21	Human	Livestock farmer	2005	Ulsan	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-3	D2	
B. abortus	#22	Human	Livestock farmer	2005	Gyeongnam	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#23	Human	Livestock farmer	2005	Chungnam	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-4	E1	
B. abortus	#28	Human	Livestock farmer	2005	Gyeonggi	5-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	G1	
B. abortus	#31	Human	Livestock farmer	2005	Daegu	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-3	E4	
B. abortus	#36	Human	Livestock farmer	2005	Chungnam	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#37	Human	Livestock farmer	2005	Daegu	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-3	E4	
B. abortus	#40	Human	Livestock farmer	2005	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-7-2-3-3-3-4	A2	
B. abortus	#42	Human	Livestock farmer	2005	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-3	E4	
B. abortus	#48	Human	Livestock farmer	2006	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-3	E4	
B. abortus	#49	Human	Livestock farmer	2006	Gyeongnam	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#50	Human	Livestock farmer	2006	Chungnam	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#53	Human	Livestock farmer	2006	Gyeonggi	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-3	D2	
B. abortus	#56	Human	Livestock farmer	2006	Gyeongnam	5-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-3	I 2	Human only
B. abortus	#60	Human	Livestock farmer	2006	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-3	E4	
B. abortus	#61	Human	Livestock farmer	2006	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#63	Human	Livestock farmer	2006	Gwangju	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	D3	
B. abortus	#68	Human	Livestock farmer	2006	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-7-2-4-3-3-3	E3	
B. abortus	#72	Human	Livestock farmer	2006	Gyeonggi	5-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-4	E5	Human only
B. abortus	#74	Human	Livestock farmer	2006	Gyeonggi	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-4	E1	
B. abortus	#79	Human	Livestock farmer	2006	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-4	E1	
B. abortus	#84	Human	Livestock farmer	2007	Gyeongnam	5-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	G1	
B. abortus	#85	Human	Livestock farmer	2007	Gyeongbuk	5-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-4	G1	
B. abortus	#87	Human	Livestock farmer	2007	Gwangju	4-4-4-5-3-4-12-3-6-21-8-6-2-3-3-3-3	D2	
B. abortus	#88	Human	Livestock farmer	2007	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-4	E1	
B. abortus	#89	Human	Veterinarian	2007	Gyeongbuk	4-4-4-5-3-4-12-3-6-21-8-6-2-4-3-3-4	E1	
B. abortus	#90	Human	Livestock farmer	2007	Daejeon	4-4-4-5-3-4-12-3-6-21-8-6-2-5-3-3-3	C5	Human only
B. abortus	#91	Human	Livestock farmer	2007	Jeonbuk	4-4-4-5-3-4-12-3-6-21-8-5-2-3-3-3-3	C1	

[a]The TRs copy numbers were arranged in the following order: Bruce 04-06-07-08-09-11-12-16-18-19-21-30-42-43-45-55-Hoof 3.
[b]Compared with 23 genotypes of animal *Brucella* isolates as reported previously [13].

clustering analyses, human isolates were divided into 12 genotypes, and a majority of the isolates coincided with genotypes of animal isolates (Table 1). The C5, E5, and I2 genotypes showing minor changes with major genotypes were only detected in humans (Table 1). However, they were not peculiar strains, but are considered to be strains originating from the same sources or strains significantly related to one another. Her *et al*. [13] reported that some of the *B. abortus* isolates that originated from the same farm at the same time were found to have two or three allelic profiles among the genetic markers with an especially high diversity index. He also inferred that the TRs copy numbers of some loci may change in the course of the adaptation in the host, *via* mouse passage experiment. Whatmore *et al*. [40] reported that strains re-isolated from pigs infected with *B. suis* showed some minor changes in TRs at the loci with high diversity index values. Such random genetic events as DNA insertions, DNA deletions,

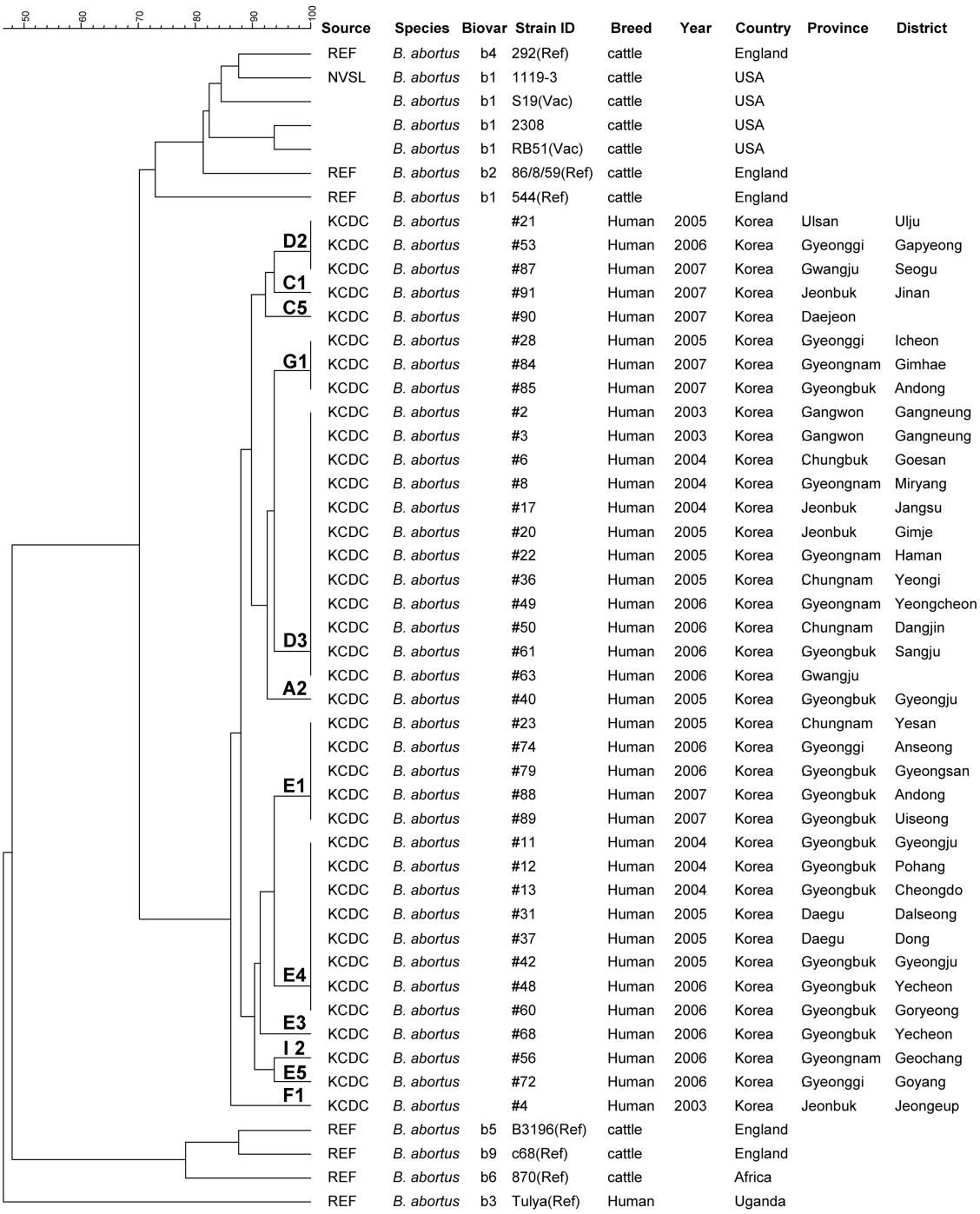

Fig. 1. Dendrogram of *B. abortus* human isolates based on the dataset of 17 loci.
Included here are 38 human isolates and 11 *B. abortus* reference or standard strains. All the isolates were confirmed to *B. abortus* strains.

point mutations of DNA, *etc*., commonly happened in the course of outbreaks or *in vitro* passages [33, 37]. During their replication within a body, resistance to any external conditions, and re-infections to other animals, mutants can be generated at the genetic sites that code for TRs. They may be promoted by changes in the natural hosts; for example, animals to humans. Similar to that, if PFGE patterns differ from outbreak patterns based on changes consistent with a single genetic event, which would result in two to three band differences, an isolate is considered to be significantly related to the outbreak strain [37].

In the maximum parsimony tree analysis, Korean *B. abortus* isolates from humans and animals were comparatively compactly clustered (Fig. 2). The seven clusters were

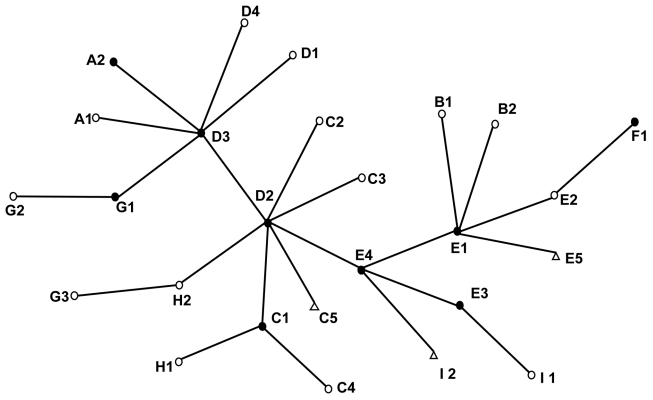

Fig. 2. Maximum parsimony tree analysis for 26 genotypes of *B. abortus* isolates from humans and animals in South Korea: "○" for animals, "●" for animals and humans, and "△" for humans only. The genotypes of the *B. abortus* human isolates were compared with 23 genotypes of *B. abortus* animal isolates reported previously [13]

detected in both humans and animals, which implies that *B. abortus* isolates from humans are significantly related to animal isolates in South Korea. Proof of this was the fact that patients were livestock farmers, workers and cohabited relatives of cattle farm with serological reactors, or neighborhood farmers near the positive farm for brucellosis (Table 1). A majority of patients were raising Korean native cattle or beef cattle, which were neglected in the eradication program before 2004. Bovine brucellosis outbreaks in Korean native cattle have only begun increasing after early 2000, and a new intensive eradication program to cover them was introduced in 2004. At the same time, there were enforcements or recommendations to test brucellosis among livestock farmers, employees, and any other epidemiological relatives engaging in the farms with reactors.

Meanwhile, a prevalence of human brucellosis, after they were first reported in 2002, showed tendency toward corresponding exactly to the number of outbreaks in cattle in South Korea [8, 19, 24, 31]. The incidences of human brucellosis by occupation during the period 2003 to 2006 were reported among 382 livestock workers (87.5%), 44 veterinarians (10%), and 11 others (2.5%) including animal by-product handlers and slaughterhouse workers [6]. In 2006, a national survey of brucellosis seroprevalence among high-risk groups including livestock-related workers was carried out [24]. For the purpose, blood samples were collected from 7,429 subjects, including 6,720 livestock workers, 335 veterinarians, and 374 artificial insemination technicians, whose seroprevalence was found to be 0.7%, 1.79%, and 0.27%, respectively. Workers and professionals engaging in slaughterhouses were also investigated in 2007. Of 1,707 subjects in total, 12 handling residual product and engaging in slaughterhouses were seropositive (0.7%). With regard to that, local control managers and laboratory workers handling *Brucella* strains will also have to be careful. It was reported that the MLVA assay could trace back to the source of a laboratory exposed to *Brucella* infections [26], and the assay is known to have a high discriminatory power for brucellosis endemic to regions in many countries [3, 10, 17, 25, 35]. It is also used to prove the geographic origin of *Brucella* strains. Al Dahouk *et al.* [2] identified 110 genotypes for *Brucella* isolates from humans, which can be categorized into 3 geographic groups: American, and West and East Mediterranean [38].

In conclusion, the MLVA assay by the use of 17 loci was confirmed to have enough discriminatory power for human and animal *Brucella* isolates in Korea, even though they did not showed high genetic diversity. This assay can also be used as part of an important epidemiological tool in controlling and preventing brucellosis. This study presents the first data for evaluating the genetic correlation of *B. abortus* isolates from humans and animals in South Korea.

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