Research Article

Assessment of PCR-Based DNA Fingerprinting Techniques as a Novel Approach for Genotyping of *Brucella* Strains in Egypt

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Abstract | This study aimed to evaluate two PCR-based DNA fingerprinting techniques: Enterobacterial repetitive intergenic consensus (ERIC) and Random amplification polymorphism DNA (RAPD), for genotyping the *Brucella* isolates; as alternative rapid tools for epidemiological tracing and investigation of brucellosis in Egypt. We recovered *Brucella* isolates (n=29) from different host species and governorates. The isolates were identified by the bacteriological and molecular techniques (AMOS-PCR) as *Brucella* (B) *melitensis* biovar 3 (n=24) and *B. abortus* bv1 (n=5). The ERIC and RAPD primers (ERIC2 and Operon 18/ RAPD4) used in this study created polymorphic band patterns in all *Brucella* isolates and the reference strains. ERIC-PCR and RAPD-PCR Dendrograms clustered the *B. melitensis* isolates into two clusters and three clusters composed of 16 and 13 genotypes with genetic similarity percentages of 62% and 54% with a diversity index of 0.82 and 0.88. Both ERIC-PCR and RAPD-PCR dendrograms clustered *B. abortus* isolates into two clusters and three genotypes with discriminatory of 0.8 and 0.72. Both ERIC and RAPD PCRs revealed 25 singleton genotypes (G) out of 35 genotypes. DNA-based typing techniques showed substantial diversity among the *Brucella* isolates. The genetic diversity among *Brucella* genotypes obtained in this study may suggest that the *Brucella* strains have been introduced into the country either intermittently or over a point of time and location. *Brucella melitensis* transmission among non-preferable hosts and passages of *Brucella* strains are likely to have resulted in such heterogeneity. We concluded that the acceptable diversity induced by ERIC-PCR and RAPD PCR allows sufficient discrimination between *Brucella* isolates. These findings recommend the application of these techniques as an inexpensive and quick tool for traceability of *B. melitensis* and *B. abortus* strains, especially in developing countries with limited resources needed for other advanced sequence-based genotyping tools. Further studies on a large scale are required to ensure the reproducibility of both techniques.

Keywords | *Brucella,* dendrogram, ERIC-PCR, genotypes, RAPD-PCR.

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INTRODUCTION

Brucellosis is a worldwide distributed zoonotic disease caused by bacteria of the genus *Brucella*, that has a serious impact on animal and human health (Cutler et al., 2005). Among the current 11 *Brucella* species, *B. melitensis*, *B. suis*, and *B. abortus* are the most virulent *Brucella* species affecting humans (Chiliveru et al., 2015; Denting-

er et al., 2015). The disease is endemic in Egypt, where three *Brucella* species namely; *B. melitensis* bv3, *B. abortus* bv1, and *B. suis* bv1 were reported through different published papers (Abdel-Hamid et al., 2017; Khan et al., 2019; Abdel-Hamid et al., 2021).

A wide range of PCR products with varying amplicon lengths are created in DNA-fingerprinting procedures such as RAPD-PCR and ERIC-PCR. These complex PCR products are used as DNA markers to identify species and can be identified by various methods (Magyar et al., 2019).

The genotyping non-sequencing-based tools such as ERIC and RAPD PCRs have been extensively used in foodborne pathogens fingerprinting (Shrivastava et al., 2018) and to a lesser extent in *Brucella* (Mercier et al., 1996; Tcherneva et al., 2000; Behroozikhah et al., 2005; Mustafa et al., 2017). The ERIC sequences are documented in an enormous number of bacterial genomes, such as the members of the family Enterobacteriaceae. In general, incomplete palindrome sequences are found in transcribed areas and linked to intergenic consensus. Furthermore, various bacterial species have varying numbers of ERIC sequence copies (Wilson et al., 2006).

ERIC is frequently used in Gram-negative enteric bacteria as one of the repetitive extragenic palindromic PCR (rep-PCR) assays that use primers targeting highly conserved repetitive sequence elements (Versalovic et al., 1991).

ERIC-PCR technique is a quick, sharp, and cost-effective fingerprint method. (Ranjbar et al., 2017). However, ERIC-PCR with ERIC1R and ERIC2 as primers proved less discriminative, permitting only genus-level differentiation with rare discrimination between individual strains of *Brucella* (Tcherneva et al., 1996). On the contrary, the ERIC-PCR has been approved to be superior in distinguishing *Brucella* strains with high discriminatory power using the same set of primers but with different cyclic parameters and annealing temperatures (Mercier et al., 1996; Mustafa et al., 2017).

The ERIC-PCR has been widely used to determine the genotypes of bacteria, including *Brucella* species, at the subspecies level. The ERIC-PCR is a reasonably simple PCR-based genotyping technology that can discriminate between individual *Brucella* strains because a pair of random primers anneal at non-specific sites at the whole genome level produces strain-specific band patterns (Bricker, 2002).

RAPD-PCR is a rapid method for the detection of genomic polymorphisms. This method relies on the use of single or multiple short oligonucleotide primers to amplify a random sequence of genomic DNA. These random primer sites' location and number vary amongst different strains of a bacterial species (Krawczyk and Kur, 2018).

RAPD-PCR is a good approach for distinguishing related bacterial species and seems to be a simple, rapid, and sensitive technique for the epidemiological investigation of brucellosis when performed under strictly established conditions. (Tcherneva et al., 2000).

ERIC-PCR and RAPD-PCR could be useful in routine epidemiological surveillance and tracing the source of fastidious microorganisms' transmission like *P. aeruginosa* (Nanvazadeh et al., 2013).

PCR-based DNA fingerprinting techniques are rapid, reliable, and cost-effective tools for genotyping many pathogens. Therefore, this study aimed to evaluate two PCR-based DNA fingerprinting techniques (RAPD and ERIC) for genotyping the *Brucella* isolates from different host species and strains as alternative rapid tools for epidemiological tracing and investigation of brucellosis in Egypt.

MATERIALS AND METHODS

Brucella isolates, phenotypic and molecular identification: Twenty-nine *Brucella* isolates were recovered from different animal species (small and large ruminants) during the period from 2014 to 2020 out of 156 lymph nodes. These *Brucella* strains were isolated from different Egyptian governorates namely; El Fayoum (n=12), Assuit (n=1), Ismailia (n=4), Gharbia (n=1), Kafr Elsheikh (n=2), Damietta (n=1), Beni-Suef (n=1), Giza (n=1), Menufia (n=2), and Dakahlia (n=4). Phenotypic characterization of the *Brucella* isolates was conducted according to Alton et al. (1988) and OIE, Terrestrial Manual (2021) in terms of colony morphology, urease, catalase, oxidase, nitrate reduction, lactose fermentation, and reaction to acriflavine (1/1000 solution) for genus identification. Furthermore, the requirement for CO_2 at initial culture, test for H_2S production, growth in presence of dyes (thionine at 1/50000 concentration; basic fuchsin at 1/50000 concentration), agglutination with A, M, and R monospecific anti-sera, and lysis by Tbilisi (Tb), Izatnagar (Iz) and R/C phages were done for conventional identification of *Brucella* species and biovars.

DNA extraction from bacterial cultures was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacturer's recommendations. Sample suspension 200 µl in phosphate buffer saline was incubated with proteinase K (10 µl) and lysis buffer (200 µl) at 56°C for 10 min. After the incubation, 200 µl of 100% ethanol were added to the lysate. The sample was

washed and centrifuged following the manufacturer's recommendations. Subsequently, the nucleic acid was eluted using 100 µl of the elution buffer. The AMOS-PCR assay was performed using *Brucella* IS711-specific forward Primer (5'-TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT-3') and two reverse primers (*B. abortus*-specific Primer 5'-GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC-3'; *B. melitensis*-specific Primer 5'-AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA-3). The AMOS-PCR cyclic conditions were performed as described by Bricker and Halling (1994).

Typing trials of ERIC-PCR and RAPD-PCR:

In order to get the optimal cyclic condition for the DNA based fingerprinting assays performed in this study (ERIC-PCR and RAPD-PCR), a different set of primers (ERIC1R/ERIC2, ERIC2, Operon18, RAPD4, and Operon18/RAPD4), different annealing temperatures, different DNA to primers concentrations, different DNA to magnesium chloride concentrations, and different extension times have been tried. The cyclic conditions concerning these trials were illustrated in Table (1).

Brucella strains and genetic diversity by using ERIC and RAPD-PCR techniques:

The 29 *Brucella* isolates (24 *Brucella melitensis* and five *Brucella abortus*) Besides, two reference strains (*B. melitensis* reference strain Ether ATCC 23458 and *B. abortus* reference strain 544 ATCC 23448) were fingerprinted by both ERIC-PCR and RAPD-PCR techniques using the ERIC2 primer (5′-AAGTAAGTGAC TGGGGTGAG-CG-3′) and Operon 18/ RAPD4 (5′-CAGCACCCAC / AAGACGCCGT-3′) respectively. The DNA concentration in nanogram(ng), primers' concentrations (pmol), and $MgCl_2$ concentration (mM), and cyclic conditions of ERIC-PCR and RAPD-PCR, are illustrated in Table 2 based on chosen trials that gave the optimal cycling conditions required for both DNA fingerprinting PCRs. Briefly, for both techniques, PCR was performed with an initial denaturation step (94 C for 5 min), followed by 40 cycles of denaturation (94 C for 1 min), annealing (ERIC, 45°C for 1 min; RAPD, 37°C for 1 min), and extension (ERIC, 72°C for 2 min; RAPD, 65 °C for 8min), followed by a final extension (ERIC, 72°C for 7 min; RAPD, 65°C for 16 min). An electrophoresis using 2% agarose gel (Applichem, Germany, GmbH) in a 1x TBE buffer was used to fractionate the PCR products. The gel was stained with ethidium bromide and observed under an ultraviolet transilluminator. A Generuler 100 bp DNA ladder (Fermentas, Thermo, Germany) was included in each gel as a molecular weight marker. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the bands' sizes were analyzed by computer software.

Genotyping and cluster Analysis of *Brucella* species fingerprinted by ERIC-PCR and RAPD-PCR assays.

The banding patterns generated by ERIC-PCR and RAPD-PCR were analyzed using GelJ version 2 (Heras et al., 2015). The dendrograms were created based on the unweighted pair group method with arithmetic mean (UPGMA) and Dice similarity coefficient. The UPGMA was used because it is considered the simplest distance-matrix method in constructing a dendrogram using uncorrected data.

Simpson's diversity indices of ERIC-PCR and RAPD-PCR:

The Simpson's diversity indices were calculated through the online website (https://www.omnicalculator.com/statistics/simpsons-diversity-index#what-is-simpsons-index) using the following formula $D = \Sigma(n_i * (n_i - 1)) / (N * (N - 1))$: where n_i= Number of strains in the *i*-th species and, N= Total number of strains in the sample's population.

RESULTS AND DISCUSSION

The conventional bacteriological typing of the 29 *Brucella* isolates resulted in confirming 24 isolates to be *B. melitensis* biovar 3 (n=24) and five isolates to be *B. abortus* biovar 1 (n=5). The *B. melitensis and B. abortus* isolates were further confirmed by AMOS-PCR assay. Amplification of bands specific to *B. melitensis* and *B. abortus*, 731bp and 498bp, were obtained (Figure 1 and 2). In Egypt, *B. melitensis* bv 3 is the most predominant and circulating *Brucella* species responsible for most human and animal cases, followed by *B. abortus* biovar 1 (Abdel-Hamid et al., 2020; Wareth et al., 2020; Hegazy et al., 2022).

PCR is an enzymatic reaction, thus the quality and concentration of the DNA template, concentrations of PCR components, and the PCR cycling conditions may significantly affect the outcome. Thus, the RAPD and ERIC techniques are notoriously laboratory dependent and need carefully developed laboratory protocols to ensure reproducibility (Mbwana et al., 2006).

Mispriming or partial priming occurs more frequently in the permissive environment for ERIC-PCR primers, leading in variable numbers and the bands' size of the amplicons. Since the annealing conditions are marginal for such semi-specific primers, primer annealing and amplicon production are readily influenced by even small variations in the assay conditions (Bricker, 2002).

Similarly, for the RAPD-PCR, minute changes in the assay-cyclic condition can easily affect the annealing efficiency and significantly alter the results (Bricker, 2002).

Table 1: The twenty-seven cycling conditions trials for ERIC and RAPD typing of *Brucella* spp.

Target gene	Trials	Primer (s)	Primer sequence (5' – 3')	DNA conc. (ng)	Primer conc. (pmol)	MgCl2 (mM)	Primary Denat.	Amplification (35 cycles)			Final extension
								Denat.	Anneal.	Exten.	
ERIC	T1	ERIC1R/ ERIC2	ATGTAAGCTCCT-GGGGATTCAC	50 ng	20 pmol	2.5 - 3 mM	94°C 5 min	94°C 1 min	52°C 1 min	72°C 8 min	72°C 16 min
			AAGTAAGTGACT-GGGGTGAGCG	50 ng	20 pmol	2.5 - 3 mM					
	T2	ERIC1R/ ERIC2	ATGTAAGCTCCT-GGGGATTCAC	50 ng	20 pmol	2.5 - 3 mM			42°C 1 min		
			AAGTAAGTGACT-GGGGTGAGCG	50 ng	20 pmol	2.5 - 3 mM					
	T3	ERIC2	AAGTAAGTGACT-GGGGTGAGCG	50 ng	20 pmol	2.5 - 3 mM			37°C 1 min		
RAPD	T4	Operon 18	CAGCACCCAC	50 ng	20 pmol	2.5 - 3 mM	94°C 5 min	94°C 1 min	37°C 1 min	72°C 2 min	72°C 7 min
	T5	RAPD4	AAGACGCCGT	50 ng	20 pmol	2.5 - 3 mM			37°C 1 min		
	T6	Operon 18/ RAPD4	CAGCACCCAC	50 ng	20 pmol	2.5 - 3 mM			40°C 1 min		
			AAGACGCCGT	50 ng	20 pmol	2.5 - 3 mM					
	T7	Operon 18/ RAPD4	CAGCACCCAC	50 ng	20 pmol	2.5 - 3 mM			45°C 1 min		
			AAGACGCCGT	50 ng	20 pmol	2.5 - 3 mM					
RAPD/ ERIC (2-Step PCR)	T8	RAPD4/ ERIC2	AAGACGCCGT	50 ng	20 pmol	2.5 - 3 mM	94°C 5 min	94°C 1 min	37°C 1 min (15 cycle)	72°C 2 min	72°C 16min
			AAGTAAGTGACT-GGGGTGAGCG	50 ng	20 pmol	2.5 - 3 mM			45°C 1 min (25 cycle)	72°C 8 min	
ERIC	**T9**	ERIC2	AAGTAAGTGACT-GGGGTGAGCG	50	20	No add	94°C 5 min	94°C 1 min	37°C 1 min	65°C 8 min	65°C 16 min
RAPD	**T10**	Operon 18/ RAPD4	CAGCACCCAC / AAGACGCCGT	10	20	2.5 - 3	94°C 5 min	94°C 1 min	45°C 1 min	72°C 2 min	72°C 7 min
	T11			10	10	2.5 - 3					
	T12			10	10	No add					
RAPD	**T13**	Operon 18/ RAPD4	CAGCACCCAC / AAGACGCCGT	10	20	5 - 6	94°C 5 min	94°C 1 min	37°C 1 min	72°C 2 min	72°C 7 min
	T14			5	20	2.5 - 3					
	T15			2.5	20	2.5 - 3					
	T16			5	20	5 - 6					
	T17			2.5	20	5 - 6					
	T18	Operon 18	CAGCACCCAC	10	20	2.5 - 3					
	T19	RAPD4	AAGACGCCGT								

ERIC	T20	ERIC2	AAGTAAGTGACT-GGGGTGAGCG	10	20	2.5 - 3	94°C 5 min	94°C 1 min	37°C 1 min	65°C 8 min	65°C 16 min
	T21			5	20	2.5 - 3					
	T22			2.5	20	2.5 - 3					
	T23			10	20	5 - 6					
	T24			5	20	5 - 6					
	T25			2.5	20	5 - 6					
RAPD	T26	Oper-on 18/ RAPD4	CAGCACCCAC / AAGACGCCGT	2	20	10	94°C 5 min	94°C 1 min	45°C 1 min	72°C 2 min	72°C 7 min
ERIC	T27	ERIC2	AAGTAAGTGACT-GGGGTGAGCG	2	20	10	94°C 5 min	94°C 1 min	37°C 1 min	65°C 8 min	65°C 16 min
RAPD	T17 (2nd)	Oper-on 18/ RAPD4	CAGCACCCAC / AAGACGCCGT	2.5	20	5 - 6	94°C 5 min	94°C 1 min	45°C 1 min	72°C 2 min	72°C 7 min

Table 2: The optimal cycling conditions for ERIC and RAPD typing of 31 *Brucella spp.* isolates (2 reference strains + 29 different isolates)

Trial	Primer (s)	Primer sequence (5' – 3')	DNA conc. (ng)	Primer conc. (pmol)	MgCl2 (mM)	Primary Denat.	Amplification (40 cycles)			Final extension
							Denat.	Anneal.	Exten.	
T17 (RAPD)	Oper-on 18/ RAPD4	CAGCACCCAC / AAGACGCCGT	2.5	20	5 - 6	94°C 5 min	94°C 1 min	45°C 1 min	72°C 2 min	72°C 7 min
T25 (ERIC)	ERIC2	AAGTAAGT-GACTGGGGT-GAGCG	2.5	20	5 - 6	94°C 5 min	94°C 1 min	37°C 1 min	65°C 8 min	65°C 16 min

Table 3: Clusters, genotypes, and diversity index of *Brucella* strains recovered from different animal species in Egypt.

Genotyping methods		Genetic similarity (%)	Clusters	Cluster size	Number of genotypes	Singleton genotypes (unique genotypes)	Simpson's diversity index
ERIC-PCR	*B. melitensis*	62	Cluster 1	5	2	1	0.82
			Cluster 2	20	14	10	
	B. abortus	63	Cluster 1	3	2	1	0.80
			Cluster 2	3	1	0	
RAPD-PCR	*B. melitensis*	54	Cluster 1	1**	1	1	0.88
			Cluster 2	5	4	3	
			Cluster 3	19	8	4	
	B. abortus	78	Cluster 1	1*	1	1	0.72
			Cluster 2	5	2	1	

**B. abortus* reference strain 544 ***B. melitensis* reference strain Ether

Therefore, it was necessary to perform several trials (27 trials) to test different annealing temperatures, different sets of primers, and different concentrations ratios between DNA, primers, and $MgCl_2$. We performed these trials to get the most optimal cyclic conditions, DNA to primers ratio for a higher capability of fingerprinting and genotyping using both techniques.

We performed the ERIC-PCR and RAPD-PCR typing based on the best trial (T) we obtained. The optimal cyclic condition was achieved in T17 for RAPD-PCR and T25 for ERIC-PCR (Table 1 and 2). In this study, Primers used for ERIC and RAPD PCRs created polymorphic band patterns in the *Brucella melitensis* strains and the reference strain (Figure 3 and 4). These bands varied in number (2 to 7 for ERIC-PCR; 2 to 8 for RAPD-PCR) and bands' sizes (229 to 1246 bp for the ERIC-PCR; 242 bp to 2456 bp for the RAPD-PCR). Corresponding pictures for both

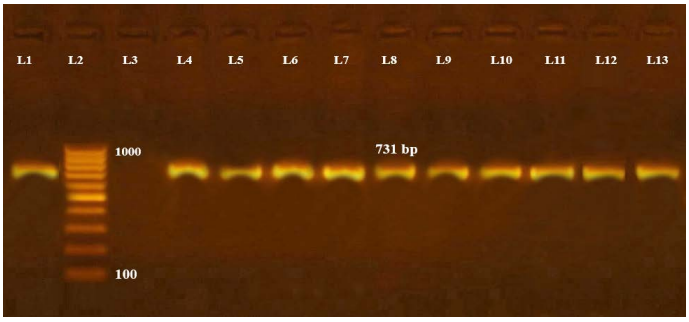

Figure 1: Differentiation of *Brucella* at species by AMOS-PCR. Lane 1 *B. melitensis* reference strain Ether; lane 2, DNA ladder; lane3, control negative; lane (4-13) *B. melitensis* field strains.

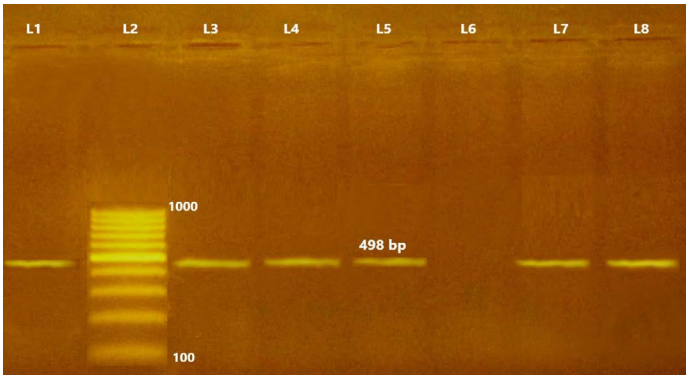

Figure 2: Differentiation of *Brucella* at species level by AMOS-PCR. Lane 1 *B. abortus* reference strain 544; lane 2, DNA ladder; lane3,4,5,7,8 *B. abortus* field strains; lane (6) control negative.

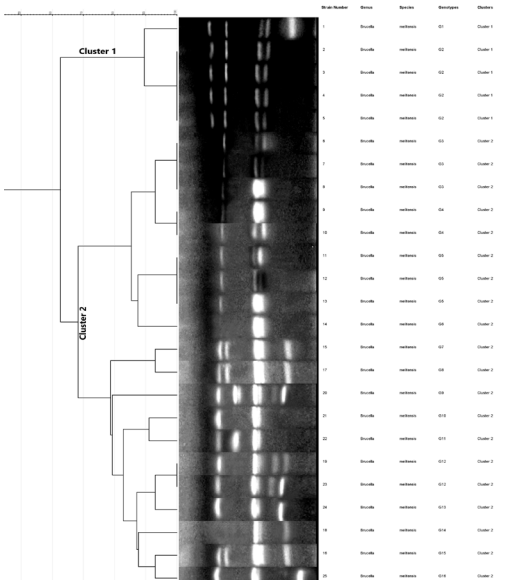

Figure 3: Cluster and genotypes analysis of ERIC-PCR fingerprints of 24 field strains of *B. melitensis* isolated in Egypt along with the reference strain Ether. Band profiles of each strain are corresponding with the lines of the dendrogram. Three major clusters 1 and 2 (similarity ~ 62%) are demarcated. The ERIC genotypes, clusters, *Brucella* species, and strain serial numbers are represented in the columns.

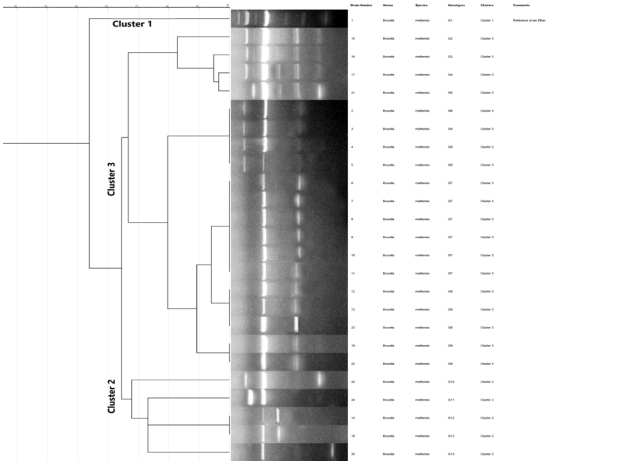

Figure 4: Cluster and genotypes analysis of RAPD-PCR fingerprints of 24 field strains of *B. melitensis* isolated in Egypt along with the reference strain Ether. Band profiles of each strain are corresponding with the lines of the dendrogram. Three major clusters 1,2 and 3 (similarity ~ 54%) are demarcated. The RAPD genotypes, clusters, *Brucella* species, and strain serial numbers are represented in the columns.

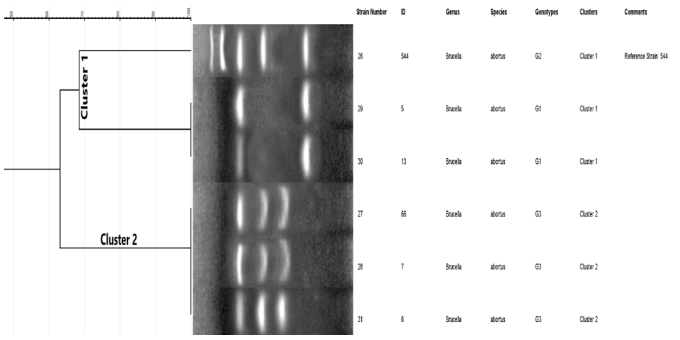

Figure 5: Cluster and genotypes analysis of ERIC-PCR fingerprints of five *B. abortus* field isolated in Egypt along with the reference strain 544. Band profiles of each strain are corresponding with the lines of the dendrogram. Two major clusters 1 and 2 (similarity ~ 63%) are demarcated. The ERIC genotypes, clusters, *Brucella* species, and strain serial numbers are represented in the columns.

techniques in *B. abortus* (Figure 5 and 6) reveal band sizes ranging from 296 to 2259bp for the ERIC-PCR. The RAPD-PCR gave bands' sizes ranging from 257 to 684 bp. Dendrograms based upon these band profiles were generated by GelJ v2 (Heras et al., 2015).

On the phylogeny based on the ERIC-PCR bands pattern of *B. melitensis* isolates (Table 3 and Figure 3), the cluster classification based on 62% similarity divided all the ERIC genotypes (G) into two major clusters, 1 and 2. Cluster 1 consisted of two ERIC genotypes (G1-G2) corresponding to 4 identical strains (G2) and one singleton genotype represented by one individual *Brucella* strain (G1). Cluster 2 comprised 14 genotypes (G3-G16) of which ten geno

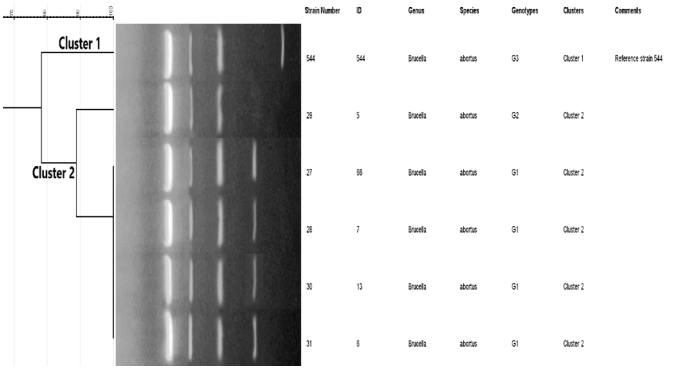

Figure 6: Cluster and genotypes analysis of RAPD-PCR fingerprints of 5 strains of *B. abortus* isolated in Egypt along with the reference strain 544. Band profiles of each strain are corresponding with the lines of the dendrogram. Two major clusters 1 and 2 (similarity ~ 78%) are demarcated. The RAPD genotypes, clusters, *Brucella* species, and strain serial numbers are represented in the columns.

types represented by individual strains (10 singleton genotypes) G3 and G5 were composed of three *Brucella* strains, and the remaining G4 and G12 had two *Brucella* strains each.

The RAPD genotypes regarding *B. melitensis* isolates are classified into slightly more clusters than which, did by the ERIC-PCR (three clusters) based on 54% genetic similarity as shown by the dendrogram (Figure 4). Cluster 1 consisted of one singleton genotype corresponding to the *B. melitensis* biovar 3 reference strain Ether (Table 3). Cluster 2 included four genotypes (G10-G13). Of these genotypes, three were represented by a single *Brucella* strain. G12 is composed of two identical *Brucella* strains. Cluster 3 consists of eight genotypes half of them are singleton genotypes.

The ERIC-PCR bands pattern-based dendrogram regarding *B. abortus* strains revealed two clusters with 63% genetic similarity (Table 3 and Figure 5). Cluster 1 contained two genotypes (G1 and G2) parallel to two identical strains (G2) and one *B. abortus* strain representing G1 (*B. abortus* reference strain 544). While cluster 2 consists of one genotype composed of three identical *B. abortus* strains (G3). The RAPD dendrogram generated for *B. abortus* was classified into two clusters with 78% genetic similarity (Table 3 and Figure 6). The *B. abortus* reference strain 544 was classified into a separate cluster (Cluster1) and singleton genotype (G3). Cluster 2 includes two genotypes (G1-G2); one is singleton genotype (G2) while, the remaining consists of four identical *B. abortus* strains.

Our findings support the findings of Mercier et al. (1996) and Mustafa et al. (2017), who found that ERIC-PCR can distinguish distinct *Brucella* strains even with a small number of polymorphic fragments. Also, supporting the findings that RAPD can distinguish between related bacterial species under controlled established conditions (Tcherneva et al., 2000).

However, typing of *B. melitensis* and *B. abortus* isolates is highly desirable for tracing the source of epidemiological outbreaks, particularly in developing countries. ERIC and RAPD PCRs can differentiate between *Brucella* at species and subspecies level for epidemiological purposes by amplifying random DNA fragments (Mercier et al., 1996; Behroozikhah, 2005).

DNA-based typing techniques used in the present study showed substantial diversity. Twenty-two singleton genotypes out of 35 genotyped were revealed by both DNA fingerprinting methods. This may suggest that the *Brucella* spp. has been widespread in point of time and location in the country. Transmission of *Brucella melitensis* among nonpreferable hosts and passages of strains probably has created such heterogeneity (Abdel-Hamid et al., 2020; Hegazy et al., 2022) This variety in genotypes detected may suggest the introduction of multiple strains to Egypt either intermittently or over a point of time.

The Simpson diversity index score has been calculated for both the ERIC and RAPD PCRs. It varies between 0 and 1 (Li et al., 2012). A high score denotes a high diversity, whereas a low score denotes a low diversity. When the diversity index is zero, there is just one species in the community (no diversity). As the genetic similarity increases, the diversity index decreases, and vice versa is true. The diversity indices of ERIC-PCR and RAPD PCR were ranged from 0.72 to 0.88, reflecting the high diversity power of the used DNA-based fingerprinting techniques used in this study and matching with the results obtained by both Mustafa et al. (2017) and Behroozikhah et al. (2005). Similarly, both techniques displayed high diversity powers in other different micro-organisms (Dorneles et al., 2014; Han et al., 2014; Purighalla et al., 2017; Shekhawat et al., 2019). This high diversity of both techniques is illustrated in Table (3). The lowest genetic similarity (54%) and high diversity power (0.88) has been achieved by the RAPD-PCR typing resulting in a grouping of *B. melitensis* strains into three clusters, which is one cluster more than that achieved by ERIC-PCR and a higher diversity index than obtained by ERIC-PCR (0.82).

Also, the high genetic similarity between *B. abortus* strains revealed by the RAPD-PCR based dendrogram reflects genetic diversity (0.72) slightly lower than attained by the ERIC-PCR (0.8).

In addition to the discriminatory power, a good DNA

typing assay should have a high level of repeatability, type ability, and stability, be simple and inexpensive, and have quick turnaround times. (Auda et al., 2017).

The sequence-based genotyping tools of *Brucella* strains are specific, well established, and reliable (Sayour et al., 2020; Holzer et al., 2021) however, these methods are time-consuming and expensive thus, could be a problem in routine epidemiological surveys and outbreak investigations especially in developing endemic country like Egypt. A recent study done on related DNA fingerprinting techniques in Egypt concluded that a combination of REP-PCR/virulence genotyping could be an affordable and applicable tool in many developing countries including Egypt (Abdel-Hamid et al., 2021), and likely did the ERIC and RAPD PCRs.

CONCLUSION

The acceptable diversity induced by ERIC-PCR and RAPD PCR allow satisfactory discrimination among individual isolates using genetic typing tools. These findings endorse the utilization of these techniques as an inexpensive and quick tool for traceability of *B. melitensis and B. abortus* strains especially in developing countries with limited resources that cannot afford the requirements to apply advanced sequence-based genotyping tools as Whole Genome Sequencing-Single Nucleotide Polymorphism (WGS-SNP) or Multiple Locus Variable-number Tandem Repeat Analysis (MLVA). However, further studies on large scale are required to confirm this and to insure the reproducibility of both techniques

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AUTHOR CONTRIBUTIONS

Conceptualization, W.E., N.H.A., M.E.R.H.; resources, All the authors; methodology, N.H.A., M.E.R.H., E.I.M.B., R.I.I; software, N.H.A.; data curation, N.H.A., M.E.R.H.; draft writing; N.H.A.; review and editing; N.H.A., M.E.R.H., E.I.M.B., R.I.I. All authors have revised and agreed to publish the manuscript in its current format.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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