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BRUCELLA MOLECULAR RECOGNITION BY MATRIX-ASSISTED LASER DESORPTION IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

By

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ABSTRACT

Brucella is an expanding genus of Gram-negative intracellular wide host ranging pathogens. This work aimed at investigating molecular recognition of *Brucella* by MALDI-TOF MS as a rapid proteomic alternative to the bacteriologic gold standard. An MSP library of 11 reference *Brucella* strains including four species was created to cover the identification of the three classic *Brucella* species reported in Egypt.A dendrogram for reference strains was plotted to analyze proteomic relations. Based on bacteriologic and proteomic biotyping of 45 field isolates, a map revealed the geographic distribution of *Brucella melitensis* and *B. abortus* from 69 unvaccinated seropositive ruminants in 12 governorates during 2015. The MALDI-TOF MS was re-evaluated as a revolutionary molecular tool for *Brucella* identification reviewing the pros and cons of the technique suggesting recent methods to tackle existing hitches. It was concluded that bacteriologic and MALDI results fully matched thanks to the limited diversity of *Brucella* isolates and the narrow MSP library. For enhanced resolution towards reliable distinction at the *Brucella* sub-species level, MALDI-TOF MS deserves selective enrichment of samples. Both *B. melitensis* Bv. 3 and *B. abortus* Bv. 1 were detected in cows and buffalo cows, while only *B. melitensis* Bv. 3 was recovered from small ruminants, a she camel and a man.

Keywords:

Brucella, MALDI-TOF MS, proteomics, dendrogram, bio typing, molecular recognition.

INTRODUCTION

Brucella is a growing genus of Gram-negative intracellular bacteria currently encompassing 12 species affecting broad livestock spectrum with a zoonotic nature. Apart from being biohazard risk group III bacteria and potential biological weapons **(OIE, 2018)**, *Brucella* members are related genetically and phenotypically rendering their subtyping a real challenge. Livestock brucellosis is an emerging disease of reproductive nature often causing abortion with extended birth-to-birth interval, retained placenta, birth of weak or dead neonates, low milk yield **(Blood *et al*.,1983)**. In males, the disease results in orchitis and epididymides. Human brucellosis is a severe debilitating febrile ailment resulting in a diversity of symptoms depending on the body organs affected with probable complications **(Madkour, 2001)**.

The bacteriologic diagnosis of brucellosis is the indomitable gold standard by far, but only with positive predictive value.False negative bacteriologic finding is probable due to *Brucella* intracellular localization, fastidiousness, slow growth and existence in samples with numbers below the detection limit of bacteriologic cultivation. Broadly speaking, molecular recognition is a diagnosis based on the detection of omics, e.g. antibodyomics, genomics, transcriptomics, proteomics, glycomics, lipidomics, metabolomics, regulomics, secretomics,etc.

The first reliable microbial classification was achieved by comparative genomic 16S rRNA sequence analysis based on phylogenetic relationship.Compared to the conserved genomics, proteomics reflects more diversity in biomarkers resulting from continuous bacterial microevolution changing the *status quo* of genetic expression to proteins **(Seng *et al*., 2009)**. The bacterial proteome varies in response to disease and the surrounding environmental conditions including exposure to antibiotics allowing for better demarcation **(Shah and Gharbia, 2017)**.Phyloproteomic clustering highly resembles taxonomy based on 16S rRNA analysis in bacterial biotyping **(Shah and Gharbia, 2017)** even at the strain level **(Culebras, 2018)**. Mass spectrometry (MS) is a group of magical analytical techniques for identifying the molecular mass as well as the chemical structure of compounds. Of the several mass spectrometry formats, the triple quadrupoles, quadrupole-time-of-flight hybrids and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) are the most common in the clinical sector.MALDI-TOF MS was first introduced by **Karas *et al.*(1987)** for molecular recognition of microorganisms based on their peptide fingerprinting.

This bioanalytical breakthrough has become one of the most captivating identification tools in the last decade. Worldwide, there are many clinical labs that use MALDI-TOF MS as a routine binomial identification tool for bacteria as a cost effective and accurate tool.

The sphere of usefulness of mass spectrometry in veterinary practice is rapidly growing **(Sayour, 2017).** The technique has several advantages over other genomic methods, such as DNA-microarrays, because fewer steps are necessary for bacterial identification, and hence, fewer errors. Another advantage of MALDI-TOF mass fingerprinting is the effortless analysis of results because extensive data processing and statistical analysis are not required, as is the case with other rapid methods for bacterial identification such as Fourier-transform infrared spectroscopy (FTIR) and DNA-microarrays. Structural analysis, powerful statistical tools and database-established bioinformatics are engaged to boost the accuracy of results. Nonstop enhancements of the *Brucella* peptide library of mass spectra **(Mesureur *et al*., 2018)** aim for better resolution at the subgenus level. , **Sayour and Sayour (2015)** using Maldi-Tof Msbio-typed 124 *Brucella* isolates from cattle, buffaloes, sheep and goats in 9 governorates. Strain-specific mass spectral projections were observed among almost all reference *Brucella* cultures used. Dendrogram clustering revealed peptide profiles of reference *Brucella* species and biovars were closer to other species than to other biovars of the same species due to the limitation of the MSP created. The aim of this investigation was to assess molecular recognition of *Brucella* by MALDI-TOF MS, using a library with more details, in comparison to classic bacteriologic identification and to study the phyloproteomic relationship among some reference *Brucella* strains including *B. melitensis*, *B. abortus* and *B. suis* known to exist in Egypt.

MATERIAL AND METHODS

2.1. *Brucella* field isolates and reference strains.

A total of 69 different tissue, milk and body fluid samples were collected from 69 unvaccinated seropositive ruminants during the year 2015. All samples were bacteriologically examined for *Brucella* microorganisms.Tested ruminants belonged to 12 governorates, *viz*. Damietta, Kafr Elsheikh, Matrouh, Ismailia, Sharkia, Dakahlia, Beheira, Monofia, Giza, Beni-Suef, Minia and Shalateen. Reference strains of *Brucella* and other bacteria are mentioned in (Table 1).

Table (1): Bacterial reference strains.

Bacterial species	Biovar	Strain	NCTC No.	Source
B. melitensis	1	16M	10094	A APHA, Weybridge, UK.
B. melitensis	1	Rev1	11362	A APHA, Weybridge, UK.
B. melitensis	3	Ether	10509	A APHA, Weybridge, UK.
B. abortus	1	544	10093	A APHA, Weybridge, UK.
B. abortus	1	1119-3	-	B NVSL, Ames, Iowa, USA
B. abortus	1	S99	11363	A APHA, Weybridge, UK.
B. abortus	1	S19	-	B NVSL, Ames, Iowa, USA
B. abortus	1	RB51	-	B NVSL, Ames, Iowa, USA
B. suis	1	1330	10316	A APHA, Weybridge, UK.
B. suis	1	2	-	A APHA, Weybridge, UK.
B. ovis	-	REO 198	-	A APHA, Weybridge, UK.
Escherichia coli	-	O157:H7	12900	A APHA, Weybridge, UK.
Staphylococcus aureus	-	-	6571	A APHA, Weybridge, UK.

A= Former Central Veterinary Laboratories, currently Animal and Plant Health Agency (APHA), New Haw, Addlestone, Surrey KT15 3NB, Weybridge, UK. B = National Veterinary Services Laboratories (NVSL), USDA, APHIS, Veterinary Services, Ames, Iowa 50010, USA.

2.2. *Brucella* phages

Table (2): *Brucella* phages for genus/ species identification of isolated brucellae.

Phage group		Propagating strain	Source
1	Tbilisi (Tb)	*Br. abortus* S19	[A] APHA, Weybridge, UK.
2	Firenze (Fi)	*Br. abortus*	[A] APHA, Weybridge, UK.
3	Weybridge (Wb)	*Br. suis* 1330	[A] APHA, Weybridge, UK.
4	Berkley (Bk_2)	*Br. melitensis* 16M	[B] NVSL, Ames, Iowa, USA
5	Rough/Canis (R/C)	*Br. canis* RM 6/66	[A] APHA, Weybridge, UK.
6	Izatnagar (Iz_1)	*Br. melitensis* 16M	[A] APHA, Weybridge, UK.

A= Former Central Veterinary Laboratories, currently Animal and Plant Health Agency (APHA), New Haw, Addlestone, Surrey KT15 3NB, Weybridge, UK. B= National Veterinary Services Laboratories (NVSL), USDA, APHIS, Veterinary Services, Ames, Iowa 50010, USA.

2.3. Chemicals and reagents.

Acetonitrile chromosolve grade was purchased from Riedel - de Haën, Germany. High purity water for HPLC was supplied from Doprogenic, Kimya, and Ankara, Turkey. Ethanol Absolute GR, and Trifluoroacetic acid CAS 76-05-1, were supplied from Sigma-Aldrich, Germany. Formic acid GR 99% was obtained from Oxford Laboratory, India. Alpha-cyano-4-hydroxy cinnamon acid (HCCA), was purchased from Bruker Daltonics, Gmbr.,Germany. Bacterial Test Standard (BTS) as reference material (peptides) for MALDI-Biotyper, Ref. no. 8256343, Lot no. 0000199130, was obtained from Bruker Daltonics Gmbr., Germany. Matrix reagent solution was prepared as saturated solution of HCCA (alpha -cyano- 4hydroxy cinnamic acid) in organic solvent (50% acetonitrile and 2.5% trifluoroacetic acid).

2.4. *Brucella* culture conditions.

Bacteriologic samples as well as *Brucella* reference strains were cultivated according to **Corbel and Banai (2005)** onto Brucella agar medium with added 5% inactivated horse serum, Oxoid microbiology product code CM0169, Oxoid Limited,Thermo Fisher Scientific Inc., UK. Slopes were incubated under CO_2 tension at 37° C for 2 days until the appearance of *Brucella* streaks.

2.5. Phenotypic identification and Biotyping.

Identification of *Brucella* genus, species and biovar was performed according to **Alton *et al.* (1988)**, **Corbel and Banai, (2005) and OIE (2018).**

2.6. Preparation of bacterial cultures for MALDI-TOF.

Preparation of bacterial cultures was performed as previously described by **Lista *et al.* (2011).** Protein was extracted from bacterial cultures under test. These extracts were spotted on the MADI-TOF target plate (MTP 384 target polished steel, Bruker Daltonics) and air dried. Subsequently, the spots were overlaid with the matrix and air dried at room temperature.

2.7. MALDI-TOF (proteomic) Biotyping.

2.7.1. Instrument conditions.

The instrument used was MALDI Microflex LT,Bruker Daltonics,Bremen, Germany.Peptide mass fingerprint product ion spectra were acquired in a linear positive mode at laser frequency of 60 Hz within a mass range of 2,000 to 20,000 Daltons. Instrument parameter settings were as follows. Ion source I at 20 kV, ion source II at 18 kV, lens at 6 kV, extraction delay time of 120 ns, initial laser power of 50%, maximal laser power of 60%, and

laser attenuation offset of 25% (range of 19%). For each spectrum, 240 laser shots in 40 shot steps from different positions of the target spot (random walk movement) were automatically acquired with AutoXecute acquisition control software (Flex control version 3.0; Bruker Daltonics, Leipzig, Germany).

2.7.2. Creation of main spectra projection (MSP).

Main spectra projection (MSP) creation was performed with a total of 88 spectra acquired for each isolate from the 11 independent reference *Brucella* strains.The quality of each spectrum was assessed with Flex analysis 3.0 software (Bruker Daltonics, Bremen, Germany).

This was performed after the raw intensity spectra had been smoothed (Savitzky Golay algorithm, five width m/z and five cycles) and baseline-subtracted (TopHat algorithm).

Mass deviation within the spectra sets was analyzed.Flat-liners and spectra with peak variations (outliers) were removed from the collection, and additional measurements were carried out to obtain 11 spectra from each of the 11 reference *Brucella* strains. Eight replicates for average of three readings for each single mass spectra from m/z 2000 to 20,000 Da were selected for each specimen to generate MSP, containing averaged masses, averaged intensities,and statistics for the reproducibility of characteristic peaks. Raw spectra were then loaded into Biotyper 2.0 (Bruker Daltonics, Leipzig, Germany), and MSP creation was carried out with the default setting of the Biotyper software. Each MSP was then assigned to its specific node on the taxonomy tree.

As an evaluation, a crosswise comparison matrix was calculated, by using the main spectra of all reference strains. In addition, to evaluate the spectral variation within the single strain, the composite correlation index (CCI) was computed by loading the raw data into the Biotyper software **(Arnold and Reilly, 1998).** In order to visualize the relationship between the MSPs, dendrogram clustering was plotted with the standard settings of Biotyper 2.0.

2.7.3. Cross identification against the created *Brucella* MSP and the Bruker bacterial library.

Before assigning the MSPs to their respective nodes on the taxonomy tree, all spectra were loaded into the Biotyper software, and identification was carried out against the MSPs available in the created library. Following the creation of 11 MSPs of reference strains, each MSP was subjected for identification, and cross matching was also performed.

For comparison of two spectra **(Karger *et al*., 2013)**, MALDI Biotyper calculates MSP-based similarity scores ranging from 0 (no similarity) to 3 (complete identity). Efficiency check of

the database search was performed using BTS. Protein extracts of the 11 reference *Brucella* strains, and *Brucella* field isolates were blind-coded, and subjected to MALDI-TOF MS identification with the automated option in the Biotyper software. For reliable results, isolates giving MALDI score less than 2.3 were retested until scoring ≥ 2.3.

RESULTS

A dendrogram based on phyloproteomic relations was plotted (Figure 1) for proteomic profile matching among *Brucella* reference strains. The distance level was inversely proportional to the correlation.

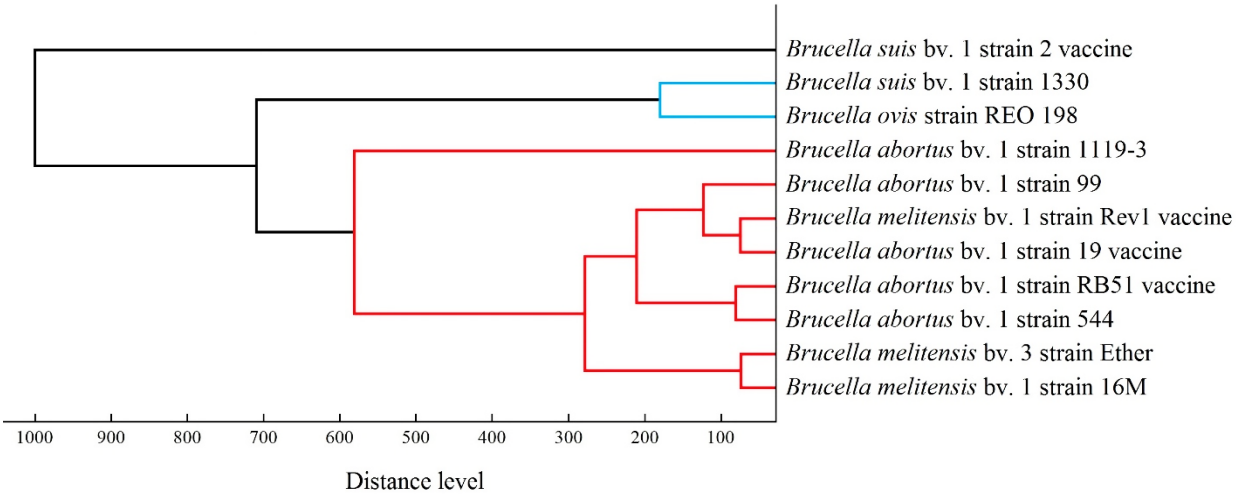

Fig. (1): Mass spectral projections (MSP) dendrogram of 11 reference *Brucella* strains.

Bacteriologic examination of samples from 69 unvaccinated seropositive ruminants revealed the detection of 45 *Brucella* field isolates from 20 cows, seven buffalo cows, seven ewes, seven goats, one she camel, one queen, one bitch and one man (Table 3). Isolates were phenotypically (Tables 3, 4 and 5) and proteomically (Table 6) identified as 29 *B. melitensis* Bv. 3 and 16 *B. abortus* Bv. 1.

Table (3): Bacteriologic genus *Brucella* recognition of 45 isolates from ruminants in 12 governorates.

Cultures		Colonial shape		Colonial phase		Micro-scopy		Differential media		Motility		Enzymes		
		Indirect inspection	Direct inspection	Acriflavine test	Crystal violet staining	Gram's method	Modified ZN	MacConkey agar	Blood agar hemolysis	at 37° C	at 22° C	Catalase	Oxidase	Nitrate reduction
Field isolates	20 from cows	Round, convex, 1-2 mm. in diameter, smooth, translucent & honey-	Round, glistening, and bluish	No agglutination	No staining	Gram-negative coccobacilli	Weak acid fast	-	-	-	-	+	+	+
	7 from buffalo													
	7 from ewes													
	7 from goats													
	1 she camel													
	1 from a queen													
	1 from a bitch													
	1 from a man													
Reference strains	*B. abortus* 544							-	-	-	-	+	+	+
	B. abortus S19							-	-	-	-	+	+	+
	B. melitensis 16M							-	-	-	-	+	+	+
	B. melitensis Rev.1							-	-	-	-	+	+	+
	B. melitensis Ether							-	-	-	-	+	+	+
	B. suis 1330							-	-	-	-	+	+	+
	B. abortus RB51		Granular, yellowish	+	purple shades			-	-	-	-	+	+	+
	B. ovis REO 198							-	-	-	-	+	-	-
	Escherichia coli					-	-	+	+	-	+	+	-	+
	S. aureus					+	-	-	+	-	-	+	-	+

Table (4): Species identification of the 45 *Brucella* field isolates recovered from ruminants in 12 governorates.

Field isolates / reference strains		Lysis by *Brucella* phage groups at RTD						Results
		1	2	3	4	5	6	
Source host	No.	Tb	Fi	Wb	Bk_2	R/C	Iz_1	
Cows	11	L	L	L	L	NL	L	*B. abortus*
	9	NL	NL	NL	L	NL	L	*B. melitensis*
Buffalo cows	3	L	L	L	L	NL	L	*B. abortus*
	4	NL	NL	NL	PL	NL	L	*B. melitensis*
Ewes	7	NL	NL	NL	L	NL	L	*B. melitensis*
Goats	7	NL	NL	NL	L	NL	L	*B. melitensis*
She camel	1	NL	NL	NL	L	NL	L	*B. melitensis*
Queen	1	L	L	L	L	NL	L	*B. abortus*
Bitch	1	L	L	L	L	NL	L	*B. abortus*
Man	1	NL	NL	NL	L	NL	L	*B. melitensis*
B. abortus 544		L	L	L	L	NL	L	
B. abortus S19		L	L	L	L	NL	L	
B. melitensis 16M		NL	NL	NL	L	NL	L	
B. melitensis Rev.1		NL	NL	NL	L	NL	L	
B. melitensis Ether		NL	NL	NL	L	NL	L	
B. suis 1330		NL	PL	L	L	NL	L	
B. abortus RB51		NL	NL	NL	NL	L	NL	
B. ovis REO 198		NL	NL	NL	NL	L	NL	
Escherichia coli		NL	NL	NL	NL	NL	NL	
S. aureus		NL	NL	NL	NL	NL	NL	

Tb = Tbilisi, Fi = Firenze, Wb = Weybridge, Bk_2 = Berkley, R/C = Rough/Canis, Iz_1 = Izatnagar, RTD = routine test dilution, L = complete lysis, PL = partial lysis, NL = no lysis.

Table (5): Biovar identification of the 45 *Brucella* field isolates recovered from ruminants in 12 governorates.

Field isolates		CO_2 demand	H_2S release	Urease test	Growth on dyes					Antisera			Biovar
					Thionin			Fuchsin					
Host	No./ species				A	B	C	B	C	A	M	R	
Cows	11 *B. abortus*	-	+	+ (2 hr.)	-	-	-	+	+	+	-	-	1
	9 *B. melitensis*	-	-	+ (18 hr.)	-	+	+	+	+	+	+	-	3
Buffalo cows	3 *B. abortus*	-	+	+ (2 hr.)	-	-	-	+	+	+	-	-	1
	4 *B. melitensis*	-	-	+ (18 hr.)	-	+	+	+	+	+	+	-	3
Ewes	7 *B. melitensis*	-	-	+ (18 hr.)	-	+	+	+	+	+	+	-	3
Goats	7 *B. melitensis*	-	-	+ (18 hr.)	-	+	+	+	+	+	+	-	3
She camel	One *B. melitensis*	-	-	+ (18 hr.)	-	+	+	+	+	+	+	-	3
Queen	One *B. abortus*	-	+	+ (2 hr.)	-	-	-	+	+	+	-	-	1
Bitch	One *B. abortus*	-	+	+ (2 hr.)	-	-	-	+	+	+	-	-	1
Man	One *B.melitensis*	-	-	+ (18 hr.)	-	+	+	+	+	+	+	-	3
B. abortus 544		+	+	+ (2 hr.)	-	-	-	+	+	+	-	-	
B. abortus S19		-	-	+ (2 hr.)	-	-	-	+	+	+	-	-	
B. melitensis 16M		-	-	+ (18 hr.)	-	+	+	+	+	-	+	-	
B. melitensis Rev.1		-	-	-	-	+	+	+	+	-	+	-	
B. melitensis Ether		-	-	+ (18 hr.)	-	+	+	+	+	+	+	-	
B. suis 1330		-	+	+(< 15	+	+	+	-	-	+	-	-	
B. abortus RB51		-	-	+ (2 hr.)	-	-	-	+	+	+	-	-	
B. ovis REO 198		-	-	-	+	+	+	+	+	-	-	+	
Escherichia coli										-	-	-	
S. aureus										-	-	-	

- = negative, + = positive, a = 1:25,000, b = 1:50,000, c = 1:100,000

Brucella isolates giving MALDI score less than 2.3 were retested until scoring more than or equal to 2.3 for reliable results. Isolated strains belonged to 12 governorates, *viz*. Damietta, Kafr Elsheikh, Matrouh, Ismailia, Sharkia, Dakahlia, Beheira, Monofia, Giza, Beni-Suef, Minia and Shalateen (Table 5) , Fig.(2).

Table (6): Origin and phenotypic/ MALDI recognition of *Brucella* field strains in 2015.

Governorate	Host	Sample	Bacteriologic/ MALDI typing	MALDI score
Damietta	C	FAC	*Brucella melitensis* bv. 3	2.51
	Q	UD	*Brucella abortus* bv. 1	2.42
	Bt	UD	*Brucella abortus* bv. 1	2.36
	C	M	*Brucella abortus* bv. 1	2.39
	C	M	*Brucella abortus* bv. 1	2.47
	C	M	*Brucella abortus* bv. 1	2.56
	C	M	*Brucella abortus* bv. 1	2.50
	C	M	*Brucella abortus* bv. 1	2.52
	C	M	*Brucella abortus* bv. 1	2.53
	BC	M	*Brucella abortus* bv. 1	2.43
	BC	P	*Brucella abortus* bv. 1	2.45
	BC	UD	*Brucella abortus* bv. 1	2.48
Kafr Elsheikh	C	SMLN	*Brucella melitensis* bv. 3	2.56
	E	SMLN	*Brucella melitensis* bv. 3	2.35
Matrouh	E	FLv	*Brucella melitensis* bv. 3	2.65
	E	FLg	*Brucella melitensis* bv. 3	2.53
	Gt	FLg	*Brucella melitensis* bv. 3	2.34
	Gt	FAC	*Brucella melitensis* bv. 3	2.35
	Gt	Sp	*Brucella melitensis* bv. 3	2.35
	Gt	Lv	*Brucella melitensis* bv. 3	2.28
	Gt	Lg	*Brucella melitensis* bv. 3	2.39
Ismailia	BC	M	*Brucella melitensis* bv. 3	2.49
	BC	M	*Brucella melitensis* bv. 3	2.60
Sharkia	C	SMLN	*Brucella melitensis* bv. 3	2.37
	E	SMLN	*Brucella melitensis* bv. 3	2.34
	C	M	*Brucella abortus* bv. 1	2.45
	C	M	*Brucella abortus* bv. 1	2.39
	C	M	*Brucella abortus* bv. 1	2.45
Dakahlia	C	M	*Brucella abortus* bv. 1	2.47
	BC	UD	*Brucella melitensis* bv. 3	2.46
	BC	Uterus	*Brucella melitensis* bv. 3	2.30
Beheira	C	Sp	*Brucella melitensis* bv. 3	2.56
	Gt	RFLN	*Brucella melitensis* bv. 3	2.37
Monofia	E	SMLN	*Brucella melitensis* bv. 3	2.55
	E	SMLN	*Brucella melitensis* bv. 3	2.41
	C	Sp	*Brucella melitensis* bv. 3	2.52
	C	M	*Brucella abortus* bv. 1	2.49
Giza	E	SMLN	*Brucella melitensis* bv. 3	2.46
	Gt	SMLN	*Brucella melitensis* bv. 3	2.35
Beni-Suef	C	SMLN	*Brucella melitensis* bv. 3	2.53
	C	SMLN	*Brucella melitensis* bv. 3	2.37
	C	RPLN	*Brucella melitensis* bv. 3	2.52
	Man	FB	*Brucella melitensis* bv. 3	2.46
Minia	C	M	*Brucella melitensis* bv. 3	2.54
Shalateen	SC	FAC	*Brucella melitensis* bv. 3	2.49

C = cow, BC = buffalo cow, E=ewe, Gt=female goat, SC = she camel, Q=queen, Bt =bitchFAC = fetal abomasal contents, UD = Uterine discharge, M= milk, P = placenta, SMLN = supramammary lymph node, RPLN = retropharyngeal lymph node. Sp = spleen, Lv = liver, Lg = lung, FB = febrile blood. For reliable results, isolates giving MALDI score less than 2.3 were retested until scoring ≥ 2.3.

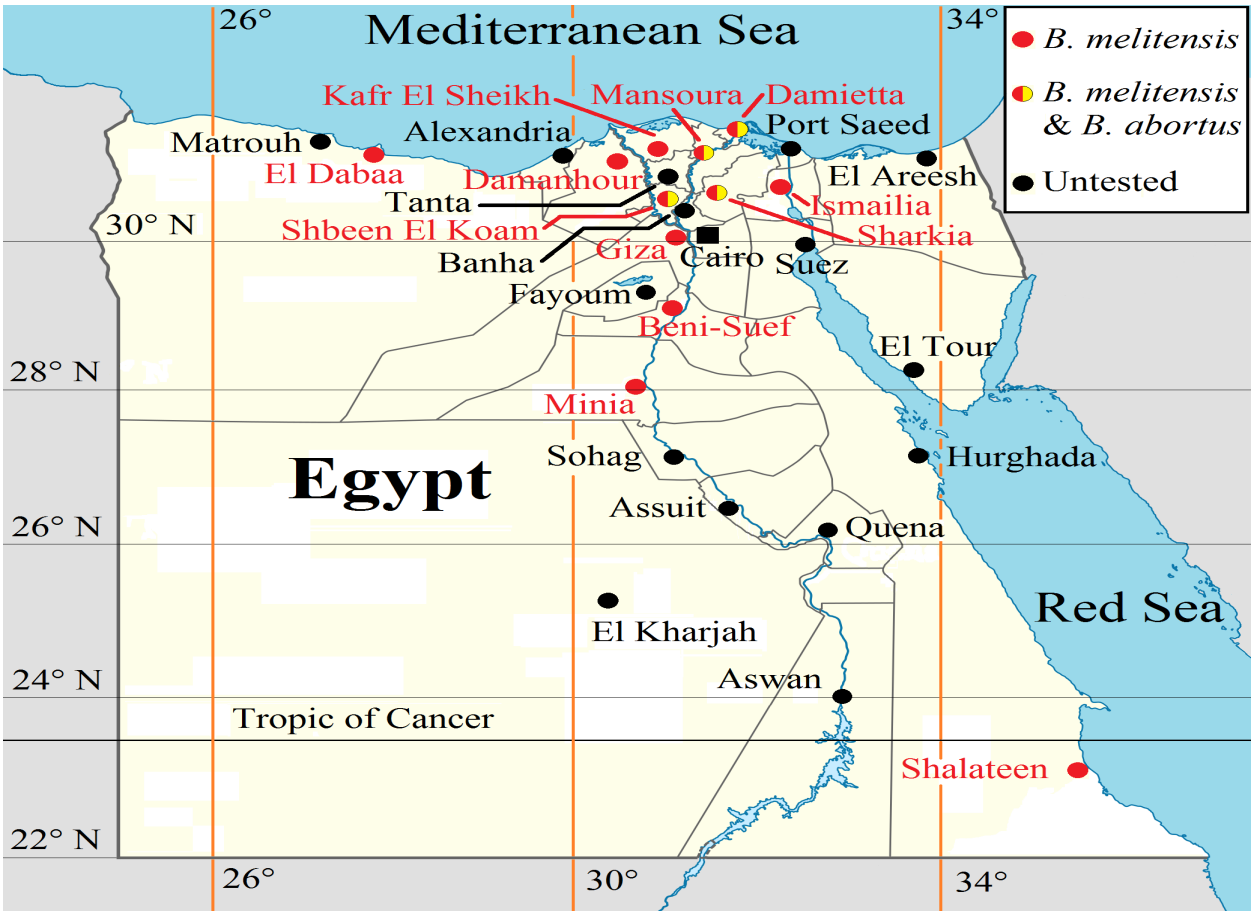

Fig. (2): Geographic distribution of *Brucella* isolates in the cities of twelve Egyptian governorates during 2015.

DISCUSSION

4.1. Taxonomic exclusivity of genus *Brucella*.

4.1.1. Taxonomic niche.

Brucella belongs to cellular organisms, superkingdom Bacteria, phylum Proteobacteria (Nitrogen-fixing bacteria), class α-Proteobacteria, subdivision α-2 Proteobacteria, order *Rhizobiales*. The phylogenetic position of *Brucella* within the alpha-proteobacteria was established on the basis of ribosomal cistron similarities and 16S rRNA sequence comparisons **(De Ley *et al.*, 1987; Moreno *et al.*, 19990; Yangi and Yamasato, 1993)**.

Brucella shares close relationships with soil organisms (e.g. *Ochrobactrum* spp.), with plant symbionts (e.g. *Rhizobium* spp.) and with phytopathogens (e.g. *Agrobacterium* spp.).

The family *Brucellaceae* includes the genera *Brucella*, *Daeguia*, *Falsochrobactrum*, *Mycoplana*, *Ochrobactrum*, *Penochrobactrum*, and *Pseudochrobactrum*. The evolution of genus *Brucella* is

thought to have originated from soil bacterial ancestors passing to fish and amphibian hosts and finally the current successors adapted to mammalian hosts **(Al Dahouk *et al*., 2017).**

4.1.2. Genus expansion.

The *Brucella* genus used to be classified into six species: *B. abortus*, *B. melitensis*, *B. suis*, *B. neotomae*, *B. ovis*, and *B. canis* based mainly on host preference, pathogenic variation and phenotypic characters **(Corbel and Brinley-Morgan, 1984)**.Since the early nineties of the last century, genus *Brucella* has been stretched out to encompass newly discovered species. These species include *B. ceti* and *B. pinnipedialis* from cetaceans and pinnipeds **(Foster *et al.*, 2007),** *B. microti* from common voles, red foxes **(Scholz *et al*., 2008)** and soil **(Scholz *et al.*, 2009),** *B. inopinata* from a breast implant of a woman infected with brucellosis **(Scholz *et al.*, 2010),** *B. papionis* from baboons **(Whatmore *et al*., 2014),** and *B. vulpis* from red foxes **(Scholz *et al*., 2016).** Lately, many atypical brucellae have been isolated.These strains did not form a distinct genetic cluster by themselves, but rather belonged to clusters including other *Brucella* species **(Al Dahouk *et al*., 2017).** Until further information becomes available about the genus members, such intermediary characters of atypical strains will temporarily suspend their affiliation to an existing species or designation as a novel species.

4.1.3. *Brucella* phenomic and genomic homogeneity.

Phenotypic variation among *Brucella* members is quantitative rather than qualitative.

This is largely dependent on colonial and microscopic morphology, biochemical characters, antigenic structure, susceptibility to bacteriophages and metabolic profiles. *Brucella* is a monophyletic genus, i.e. species descended from a common evolutionary ancestor (s). Although *Brucella* species can be differentiated by phenotypic tests, these species reveal a high degree of DNA homology. Genetically speaking, the genus *Brucella* is monospecific **(Verger *et al*., 1985)** as compared to other bacterial genera.Many genotyping techniques have been used to seek significant DNA variation within the genus *Brucella* to justify the current classification and to fine distinction of brucellae at the strain level for epidemiological tracing of infection. Multiplex PCR assay (Bruce-ladder) aided by bacteriological identification can have the job done. DNA sequence variations known as single-nucleotide polymorphisms (SNPs) are useful genetic markers because of their quantity and stable inheritance over generations. Real-time PCR assay based on SNP differentiation of clade scan **(Foster *et al*., 2012;Janowicz *et al*., 2018)** can provide a rapid and highly sensitive method of differentiating *Brucella* species

especially for clinical applications. Being dependent on specific DNA sequences, PCR still needs further inter-lab standardization **(Yu and Nielsen, 2010).**

4.2. *Brucella* proteomic architecture.

The *Brucella* cell surface involves a cell envelope surrounding the cytoplasm. The cell envelope entails an outer membrane, a periplasmic space and an inner membrane. The outer membrane encompasses lipopolysaccharide (LPS), outer membrane proteins (OMPs) and free lipids **(Moriyón and López-Goñi, 1998).** The smooth LPS is formed of an outmost O-polysaccharide chain topping the vast majority of the surface and connected to an inner core oligosaccharide and an innermost lipid an anchored in the outer membrane.

4.3. Proteomic recognition of *Brucella* by MALDI-TOF MS.

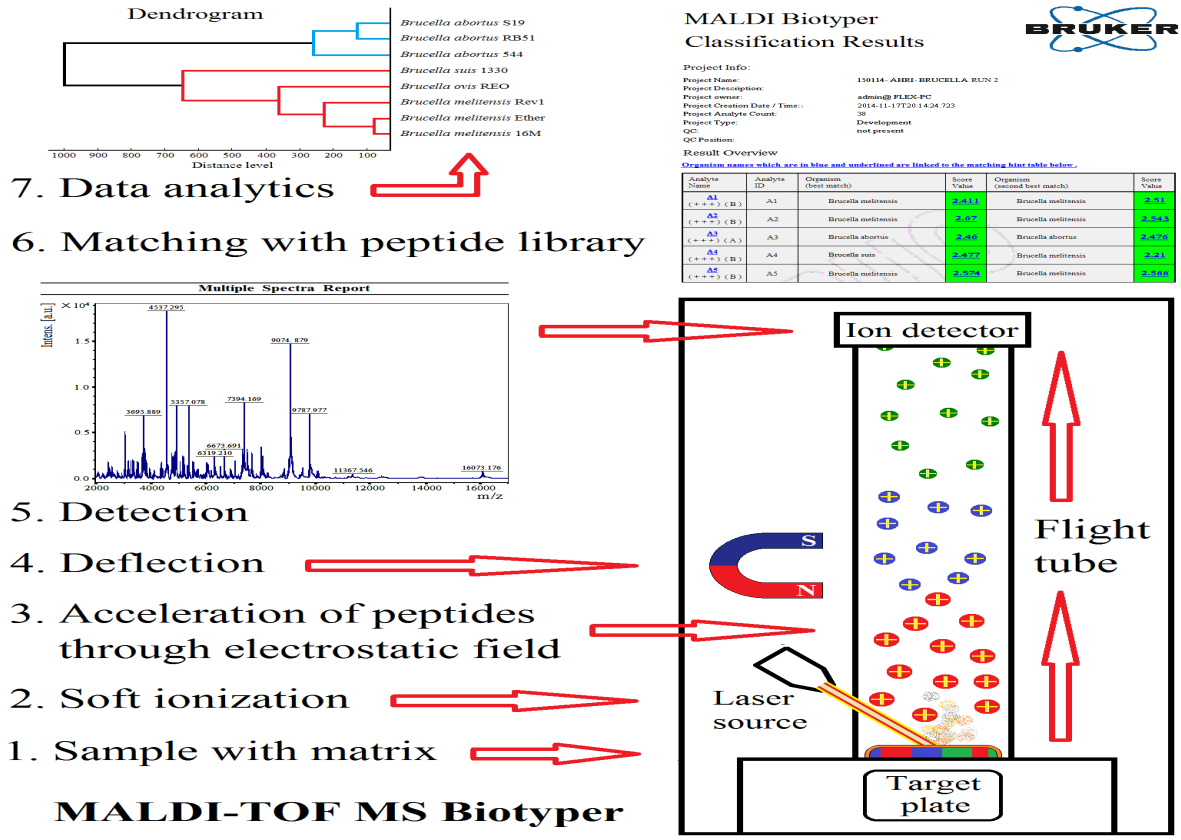

Fig. (3): The concept of MALDI-TOF MS molecular recognition of *Brucella.*

The MALDI-TOF MS principle includes mixing of the matrix material with bacterial sample. Soft ionization of sample molecules takes place by bombarding with a laser beam transferring protons from the matrix. Molecules are ionized and desorbed to form a plume (cloud) of gaseous ions. This plume of ions is detected by a TOF (time-of-flight) analyzer, in which accelerated ions obtain a mass/charge-dependent velocity when flying through an electrostatic field in the flight tube. After acceleration, the ions drift through a field-free region where their different velocities separate them as they reach the detector. Separated ions are then counted and converted into a signal for each *m/z* value proportional to the number of ions present.

The resulting output is a mass spectrum indicative of molecular masses of ions in the original plume. The technique links the mass of an ion (expressed as mass-over-charge ratio, m/z) to its flight time to the detector Fig. (3). Joint with reference peptide databases and advanced software, MALDI-TOF MS has taken microbial biotyping to the next level.

4.4. The use of mass spectrometry technology (MALDI-TOF) in clinical microbiology.

Bacterial identification is reached by either identifying biomarker ion masses related with theoretically determined protein masses obtained from databases or by comparing the whole spectrum to a spectral reference database **(Van Baar, 2000)**. It is not necessary to identify the proteins but just to determine a number of characteristic peaks representative of certain bacterial genus and/or species. The mass range of 2000 to 20,000 Da generated high inter-specific variability and simultaneously promoted high intra-specific similarity for most bacterial species. In lower mass ranges, e.g., 500-2500 Da, the variability between mass fingerprints of different species can be either too low for species differentiation, or mass fingerprints of closely related isolates can be very different. Speciation generally focuses on conserved mass spectral peaks across all isolates from a bacterial species, but subspecies typing depends on the matching of exclusive peaks using extensive peptide library and bioinformatics analysis **(Culebras, 2018).** Distinctive mass spectral peaks associated with *Brucella* strains have been reported **(Sayour and Sayour, 2015).** Despite the availability of genomic and proteomic data for strain-level demarcation of many bacteria, proteomic data for high-fidelity *Brucella* strain typing is about to be completed. Although promising, the performance robustness, resolution, and discriminatory power of MALDI-typing need to be improved. This could be achieved by one or more of the following methods; modification of the bacterial growth conditions, use of alternative protein extraction methods, use of specific

bacterial extracts, use of other matrix materials, and changing the mass range or the post-handling of mass spectra.Unfortunately, mass spectra may present an intrinsic variability in peak intensity and/or peak location associated with independent acquisitions in time and in different devices. This is possibly associated with individual instrument variation and/or regular wear over time of laser intensity, laser life, and detector responsiveness hindering the comparison of independent data sets. To evade such variations, technical steps such as growth, sample preparation, and device configuration must be performed by professional analysts using quality-controlled consumables.

4.5. Selective enrichment methods of samples prior to proteomic fingerprinting.

Protein biomarkers of diagnostic significance usually exist at very low concentration (ppb) in complex matrices, making standard targeted detection highly challenging requiring prior affinity separation techniques **(Ahmad-Tajudin *et al*., 2014).** Phosphorylation is one of the posttranslational modifications of proteins for selective enrichment.

The resultant phosphopeptides are further separated by immobilized metal ion affinity chromatography (IMAC) beads with ferric ions and titanium oxide **(Zou *et al*.,2000);Yue *et al*.,2015)**. Tyrosine-phosphorylated peptides resulting from tryptic digestion usually fail chemo- or immunoaffinity enrichments and therefore remain undetected. Natural antibodies are costly with short-term stability to be used in immunoaffinity for selective enrichment. Alternatively, aptamers are tolerant to trypsin digestion resulting in no background interference **(Lee *et al*.,2014)**. Several attempts of solid-phase immobilized aptamer platforms for selective protein identification by mass spectrometry have been fruitful **(Dick and McGown, 2004; Cole *et al*., 2007)**. Recent developments involve the use of MIPs selective for tyrosine-phosphorylated peptides to solve this problem offering better sensitivity and resolution **(Jagadeesan *et al*., 2015).** Selective enrichment by magnetic imprinted nanoparticles was applied for desalting of proteins **(Wan *et al*.,2015).** Phosphate-imprinted mesoporous silica nanoparticles (MSNs) were also employed as a sorbent for selective enrichment of phosphopeptides **(Chen *et al*., 2016).** Molecularly imprinted poly-acrylamido-derivative nanogels were used for targeted selective enrichment as well **(Bertolla *et al*., 2017)**. Solid phase extraction by MIPs of different materials were screened for best fit to certain peptide target under optimal conditions **(Jagadeesan *et al*., 2017).** Prior integrated selective enrichment target (ISET) in the form of solid-phase micro extraction of samples **(Ekstrom**

***et al*., 2007)** can be used to enhance MALDI-TOF MS resolution/sensitivity for better within genus *Brucella* demarcation. Table (6) sums up the pros and cons of MALDI-TOF MS biotyping with special reference to *Brucella*.

Table (6): Summarized pros and cons of *Brucella* MALDI-TOF MS biotyping

Criteria	Pros	Cons
Rapidity	**Within minutes automated highly sensitive culture ID**	**High-quality sample preparation needed to avoid noise and strict standardization necessary to avoid mass spectral variation**
Accuracy	**Higher than that of biochemical systems or 16S rRNA sequencing**	**Safe *Brucella* genus ID, but subgenus ID is prone to high intra-generic similarity and the versatility of the library**
Typing flexibility	**Available sample backtrack for further analysis and possibility to build custom libraries for various purposes**	**Selective enrichment of sample needed for mapping protein posttranslational modifications**
Peptide fingerprinting	**Ideal for already confirmed/ sequenced peptides**	**Not the technique of choice for detection/ identification of new peptides**
Cost	**Two-thirds less than bacteriology considering materials and staff**	**Maintenance and consumables are inexpensive, but the cost of the spectrometer is high**
Usefulness	**First-line epidemiological typing tool for instant application of infection control measures**	
	Phyloproteomic dendrograms may allow future strain distinction	

In the current investigation, a library for *Brucella* recognition by MADI-TOF MS was created based on the proteomic profiles of 11 *Brucella* reference strains. This MSP creation broadly covers strain varieties of the first 3 classic *Brucella* species that have been reported in Egypt **(Sayour and Sayour, 2015)** in addition to the live *B. abortus* and *B. melitensis* vaccines used for livestock immunization. A dendrogram based on phyloproteomic relations was plotted Fig. (1) for proteomic profile matching among *Brucella* reference strains. The distance level is inversely proportional to the correlation. All in all, strains of *B. abortus* and *B. melitensis* were proteomically related with *B. abortus* strain 1119 - 3 relatively distant. High proteomic resemblance existed between the following pairs of strains, the vaccines Rev1 and S19, *B. abortus* Bv. 1 strains RB51 and 544, and *B. melitensis* biovars 1 and 3. The high similarity between the different species Rev1 and S19 can be attributed to the dual presence of identical peptides with m/z ratios of 3024, 8036, 9074, 11369 and 16073 **(Sayour and Sayour, 2015)**. Peptide matching of *B. abortus* Bv. 1 colonial phase differing strains, *viz*. the

smooth 544 and the rough RB51 confirmed the fact that, the latter was a rifampicin and penicillin dependent mutant of the mother strain *B. abortus* bv. 1 strain 2308 **(Schurig *et al*., 1991)** was mainly different from the CO_2 requiring strain 544 in its CO_2 independence **(OIE Terrestrial Manual, 2018)**. Such matching was revealed by a replica of peptides existing in both strains of 2585, 3024, 3757, 4896, 6042, 7511, 9074, 9787 and 16073 m/z ratios **(Sayour and Sayour, 2015)**. The highly matching proteomes of *B. melitensis* biovars 1 and 3 reflect the fact that, the biovars of *B. melitensis* are actually serovars that are biologically alike **(Corbel and Banai, 2005)**. This *B. melitensis* Bv. 1/3 peptide fingerprinting homology was reflected by many identical MS peaks at m/z ratios of 2585, 3695, 4538, 6672, 7393, 9074, 9787 and 16073 **(Sayour and Sayour, 2015)**. The smooth *B. suis* Bv. 1 strain 1330 and the rough *B. ovis* strain Reo had close phyloproteomic relationship. This proteomic similarity may be attributed to the presence of common peptides with MS peaks of 2426, 2585, 3757, 6672 and 9787 Da **(Sayour and Sayour, 2015)**. The vaccinal strain 2 of *B. suis* Bv. 1 expectedly differed from *B. suis* bv. 1 strain 1330 as the former had vaccine markers.

Bacteriologic examination of samples from 69 unvaccinated seropositive ruminants revealed the detection of 45 *Brucella* field isolates from 20 cows, 7 buffalo cows,7 ewes, 7 goats, a she camel, a queen, a bitch and a man (Table 2). Isolates were phenotypically (Tables 2, 3 and 4) and proteomically (Table 5) identified as 29 *B. melitensis* Bv. 3 and 16 *B. abortus* Bv. 1. Isolates giving MALDI score less than 2.3 were retested until scoring more than or equal to 2.3 for reliable results. Isolated strains belonged to 12 governorates, *viz*. Damietta, Kafr Elsheikh, Matrouh, Ismailia, Sharkia, Dakahlia, Beheira, Monofia, Giza, Beni-Suef, Minia and Shalateen (Table 5) , Fig.(2). All the aforementioned governorates had the predominant *B. melitensis*. Four governorates namely Damietta, Dakahlia (Mansoura city), Shatkia and Monofia **(Shbeen El-Koam)** additionally had *B. abortus* Bv.1. *B. abortus* was associated with intensive dairy farms common in those Nile Delta governorates. Previous isolation of *B. abortus* from bovine animals in those four Delta governorates was reported **(Sayour and Sayour, 2015; Wareth *et al*., 2016; El-Diasty *et al*., 2018).**

CONCLUSION

Under conditions of this investigation, it was concluded that:

- Both *B. melitensis* Bv. 3 and *B. abortus* Bv. 1 were detected in cows and buffalo cows, while only *B. melitensis* Bv. 3 was recovered from small ruminants, a she camel and a man.
- Bacteriologic and MALDI biotyping results fully matched thanks to the limited diversity of *Brucella* isolates including only two species and the narrow MSP library.
- For better distinction at the *Brucella* sub-species level, MALDI-TOF MS deserves selective enrichment of samples.

EFERENCES

Ahmad-Tajudin, A.; Adler, B.; Ekström, S.; Marko-Varga, G.; Malm, J.; Lilja, H. and Laurell, T. (2014): MALDI-target integrated platform for affinity-captured protein digestion. Anal. Chim. Acta, 807: 1-8. Doi: 10.1016/j.aca.2013.08.051.

Al Dahouk, S.; Köhler, S.; Occhialini, A.; de Bagüés, M. P. J.; Hammer, J. A.; Eisenberg, T.; Vergnaud, G.; Cloeckaert, A.; Zygmunt, M. S.; Whatmore, A. M.; Melzer, F.; Drees, K. P.; Foster, J.T.; Wattam, A. R. and Scholz, H. C. (2017): *Brucella* spp. of amphibians comprise genomically diverse motile strains competent for replication in macrophages and survival in mammalian hosts. Scientific Reports, 7: 44420. Doi: 10.1038/srep 44420.

Alton, G. G.; Jones, L. M.; Angus, R. D. and Verger, J. M. (1988): Techniques for the brucellosis laboratory. Institut National de la Recherche Agronomique, Paris, France.

Arnold, R. J. and Reilly, J.P.(1998): Fingerprint matching of *E. coli* strains with matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry of whole cells using a modified correlation approach. Rapid Commun. Mass Spectrom, 12 (10): 630 - 636.

Bertolla, M.; Cenci, L.; Anesi, A.; Ambrosi, E.; Tagliaro, F.; Vanzetti, L.; Guella, G. and Bossi, A. M. (2017): Solvent-responsive molecularly imprinted anogels for targeted protein analysis in MALDI-TOF Mass Spectrometry. ACS Appl. Mater. Interfaces, 9 (8): 6908-6915. Doi: 10.1021/acsami.6b16291.

Blood, D.C.; Henderson, I. A. and Arundel, J. H. (1983): A textbook of the disease of cattle, sheep, pigs, goats and horses. Sixth Edition, English Language Book Society and Bailliere Tindall, London.

Chen, Y.; Li, D.; Bie, Z.; He, X. and Zhen Liu, Z. (2016): Coupling of phosphate-imprinted mesoporous silica nanoparticles-based selective enrichment with MALDI-TOF MS for highly efficient analysis of protein phosphorylation. Anal. Chem., doi: 10.1021/acs.analchem.5b04343 ,2015.

Cole, J. R.; Dick, L. W. Jr.; Morgan, E. J. and McGown, L. B. (2007): Affinity capture and detection of immunoglobulin E in human serum using an aptamer-modified surface in matrix-assisted laser desorption/ionization mass spectrometry. Anal. Chem., 79 (1): 273-279.

Corbel, M. J. and Banai, M. (2005): Genus *Brucella* Mayer and Shaw 1920. In: D. J. Brenner; N. R. Krieg and J. T. Staley (Editors). Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 2: The *Proteobacteria* (Part C), Springer, USA. pp 370 -386.

Corbel, M. J. and Brinley-Morgan, W. J. (1984): Genus *Brucella*. Meyer and Shaw 1920.In: KREIG, N. R. and HOLT, J. G. (Editors).Bergey's Manual of Systematic Bacteriology, Vol. 1, Baltimore, Williams and Wilkins Co., Baltimore, USA. pp. 377-388.

Culebras, D. E. (2018): Application of MALDI-TOF MS in bacterial strain typing and taxonomy. In: The use of mass spectrometry technology (MALDI-TOF) in clinical microbiology.Chapter 15, pp. 213-233, Academic Press. Doi: 10.1016/B978-0-12-814451-0.00015 - 0.

De Ley, J.; Mannheim, W.; Segers, P.; Lievens, A.; Denijn, M.; Vanhoucke, M. and Gillis, M. (1987): Ribosomal ribonucleic acid cistron similarities and taxonomic neighborhood of *Brucella* and CDC group VD. Int. J. Syst. Bacteriol., 37: 35 - 42.

Dick, L. W. Jr. and McGown, L. B. (2004): Aptamer-enhanced laser desorption/ionization for affinity mass spectrometry. Anal. Chem., 76 (11): 3037-3041.

Ekstrom, S.; Wallman, T. L. L.; Helldin, G.; Nilsson, J. and Marko-Varga, G. (2007): Polymeric integrated selective enrichment target (ISET) for solid-phase-based sample preparation in MALDI–TOF MS. J. Mass Spectrom. 42: 1445 -1452.

El-Diasty, M.; Wareth, G.; Melzer, F.; Mustafa, S.; Sprague, L. D. and Neubauer, H. (2018): Isolation of *Brucella abortus* and *Brucella melitensis* from seronegative cows is a serious impediment in brucellosis control. Vet. Sci., 5 (28). Doi: 10.3390/vetsci5010028.

Foster, G.; Osterman, B. S.; Godfroid, J.; Jacques, I. and Cloeckaert, A. (2007): *Brucella ceti* sp. Nov. and *Brucella pinnipedialis* sp. Nov. For *Brucella* strains with cetaceans and seals as their preferred hosts. Int. J. Syst. Evol. Microbiol, 57 (11): 2688-2693. Doi: 10.1099/ijs.0.65269-0.

Foster, J. T.; Price, L. B.; Beckstrom-Sternberg, S. M.; Pearson, T.; Brown, W. D.; Kiesling, D. M.; Allen, C. A.; Liu, C. M.; Beckstrom-Sternberg, J.; Roberto, F. F. and Keim, P. (2012): Genotyping of *Brucella* species using clade specific SNPs. BMC Microbiol, 12: 110. Doi: 10.1186/1471-2180-12-110.

Jagadeesan, K. K.; Rossetti, C.; Abdel Qader, A.; Reubsaet, L.; Sellergren, B.; Laurell, T. and Ekström, S. (2017): Filter plate-based screening of MIP SPE materials for capture of the biomarker pro-gastrin-releasing peptide.SLAS Discovery,22 (10):1253 - 1261.Doi: 10.1177/2472555216689494.

Jagadeesan, K. K.; Wierzbicka, C.; Laurell, T.; Sellergren, B.; Shinde, S. and Ekström, S. (2015): Multiplexed MALDI-MS arrays for screening of MIP solid phase extraction materials. J. Chromatogr. B, http://dx.doi.org/10.1016/j.jchromb.2015.10.033.

Janowicz, A.; De Massis, F.; Ancora, M.; Cammà, C.; Patavino, C.; Battisti, A.; Prior, K.; Harmsen, D.; Scholz, H.; Zilli, K.; Sacchini, L.; Di Giannatale, E. and Garofolo, G. (2018): Core genome multilocus sequence typing and single nucleotide polymorphism analysis in the epidemiology of *Brucella melitensis* infections. J. Clin. Microbiol, 56 (9): e00517-18. Doi: 10.1128/JCM.00517-18.

Karas, M.; Bachmann, D.; Bahr, U. and Hillenkamp, F. (1987): Matrix-assisted ultraviolet laser desorption of non-volatile compounds. Int. J. Mass Spectrom. Ion. Proc., 78: 53 - 68.

Karger, A.; Tomaso, H.; Melzer, F.; Timke, M.; Neubauer, H.; Bettin, B.; Al Dahouk, S.; Kostrzewa, M.; Nöckler, K. and Hohmann, A. (2013): Interlaboratory comparison of intact-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry. Results for identification and differentiation of *Brucella* spp. J. Clin. Microbiol, 51 (9): 3123-3126.

Lee, S. J.; Malm, J.; Adler, B.; Ekstrom, S.; Rezeli, M.; Vegvari, A.; Park, J.-W. and Laurell, T. (2014): Aptamer/ISET-MS: a new affinity-based MALDI technique for improved detection of biomarkers. Anal. Chem., 86: 7627-7634.

Lista, F.; Reubsaet, F. A. G.; De Santis, R.; Parchen, R. R.; de Jong, A. L.; Kieboom, J.; van der Laaken, A. L.; Voskamp-Visser, I. A. I.; Fillo, S.; Jansen, H. J.; Van der Plas, J. and Paauw, A. (2011): Reliable identification at the species level of *Brucella* isolates with MALDI-TOF-MS. BMC Microbiology, 11: 267. Doi: 10.1186/1471-2180 - 11 - 267.

Madkour, M. M. (2001): Madkour's brucellosis. Second Edition, Springer-Verlag Berlin Heidelberg New York. Doi: 10.1007/978-3-642-59533-2.

Mesureur, J.; Arend, S.; Cellière, B.; Courault, P.; Cotte-Pattat, P.; Totty, H.; Deol, P.; Mick, V.; Girard, V.; Touchberry, J.; Burrowes, V.; Lavigne, J.; O'Callaghan, D.; Monnin, V . and Keriel, A. (2018): A MALDI-TOF MS database with broad genus coverage for species-level identification of *Brucella.* PLoS Negi. Trop. Dis., 12 (10): e0006874. https://doi.org/10.1371/journal.pntd.0006874.

Moreno, E.; Stackebrandt, E.; Dorsch, M.; Wolters, J.; Busch, M. and Mayer, H., (1990): *Brucella abortus* 16S rRNA and lipid A reveal a phylogenetic relationship with members of the alpha-2 subdivision of the class Proteobacteria. J. Bacteriol., 172: 3569 -3576.

Moriyón, I. and López-Goñi, I. (1998): Structure and properties of the outer membranes of *Brucella abortus* and *Brucella melitensis*. *Internnatl. Microbiol,* 1: 19 - 26.

OIE, Manual of Diagnostic tests and Vaccines for Terrestrial Animals (2018): Chapter 2.1.4. Brucellosis (*Brucella abortus*, *B. melitensis* and *B. suis*). Version adopted in May 2016. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.04_BRUCELLOSIS.pdf.

Sayour, H. (2017): Tandem mass spectrometry as a tool for tailoring animal disease diagnostics based on biomarker discovery: Molecular imprinting approach. Fifth International Conference on Current Trends in Mass Spectrometry and Chromatography, Atlanta, USA, J. Chromatogr. Sep. Tech., 8 (6): doi: 10.4172/2157-7064 - C1 - 035.

Sayour, A. E. and Sayour, H. E. (2015): Binomial identification of *Brucella* isolates by MALDI-TOF mass spectrometry. Second Scientific Conference of Food Safety and Technology, Animal Health Research Institute, pp. 28 - 49.

Scholz, H. C.; Hubalek, Z.; Nesvadbova, J.; Tomaso, H.; Vergnaud, G.; Le Flèche, P.; Whatmore, A. M.; Al Dahouk, S.; Krüger; M.; Lodri, C. and Pfeffer, M. (2009): Isolation of *Brucella microti* from soil. Emerg. Infect. Dis., 14 (8): 1316 -1317.

Scholz, H. C.; Nöckler, K.; Göllner, C.; Bahn, P.; Vergnaud, G., Tomaso, H. Al Dahouk, S.; Kämpfer, P.; Cloeckaert, A.; Maquart, M.; Zygmunt, M. S.; Whatmore, A. M.; Pfeffer, M.; Huber, B.; Busse, H. J. and De, B. K. (2010): *Brucella inopinata* sp. Nov., isolated from a breast implant infection. Int. J. Syst. Evol. Microbiol, 60 (4): 801-808. Doi: 10.1099/ijs.0.011148-0.

Scholz, H. C.;Revilla-Fernández S.; Al Dahouk, S.; Hammerl, J. A.; Zygmunt, M. S.; Cloeckaert, A.; Koylass, M.; Whatmore, A. M.; Blom, J.; Vergnaud, G.; Witte, A.; Aistleitner, K. and Hofer, E. (2016): *Brucella vulpis* sp. nov., isolated from mandibular lymph nodes of red foxes (*Vulpes vulpes*). Int. J.Syst. Evol. Microbiol, 66 (5):2090-2098.Doi: 10.1099/ijsem.0.000998.

Scholz, H. C; Hofer, E.; Vergnaud, G.; Le Fleche, P.; Whatmore, A. M.; Al Dahouk, S.; Pfeffer, M.; Krueger, M.; Cloeckaert, A. and Tomaso, H. (2008): Isolation of *Brucella microti* from mandibular lymph nodes of red foxes, *Vulpes* in lower Austria. Vector Borne Zoonotic Dis., 9 (2): 153-156. Doi: 10.1089/vbz.2008.0036.

Schurig, G. G.; Roop, R. M. 2nd; Bagchim, T.; Boyle, S.; Buhrmanm D. and Sriranganathan, N. (1991): Biological properties of RB51; a stable rough strain of *Brucella abortus*. Vet. Microbiol, 28 (2): 171-188.

Seng, P.; Drancourt, M.; Gouriet, F.; La Scola, B.; Fournier, P. E.; Rolain, J. M. and Raoult, D. (2009): Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Infect. Dis., 49 (4): 543-551. Doi: 10.1086/600885.

Shah,H.N.and Gharbia,S.E.,Editors (2017):MALDI-TOF and tandem MS for clinical microbiology. John Wiley and Sons Ltd., UK.

Van Baar, B. L. M. (2000): Characterisation of bacteria by matrix-assisted laser desorption/ionisation and electrospray mass spectrometry. FEMS Microbiology Reviews, 24: 193 -219. Doi: 10.1111/j.1574 - 6976.2000.tb00539.x.

Verger, J. M.; Grimont, F.; Grimont, P. A. D. and Grayon, M. (1985): *Brucella*, a monospecific genus as shown by deoxyribonucleic acid hybridization. Int. J. Syst. Bacteriol, 35 (3): 292-295.

Wan, W.; Han, Q.; Zhang, X.; Xie, Y.; Sun, J. and Ding, M. (2015): Selective enrichment of proteins for MALDI-TOF MS analysis based on molecular imprinting. Chem. Commun., 51 (17): 3541. Doi: 10.1039/c4cc10205c.

Wareth, G.; Melzer, F.; El-Diasty, M.; Schmoock, G.; Elbauomy, E.; Abdel-Hamid, N.; Sayour, A. and Neubauer, H. (2016): Isolation of *Brucella abortus* from a dog and a cat confirms their biological role in re-emergence and dissemination of bovine brucellosis on dairy farms in Egypt. Transboundary and Emerging Diseases. Doi: 10.1111/tbed.12535.

Whatmore, A. M.; Davison, N.; Cloeckaert, A.; Al Dahouk, S.; Zygmunt, M. S.; Brew, S. D.; Perrett, L. L.; Koylass, M. S.; Vergnaud, G.; Quance, C.; Scholz, H. C.; Dick E. J. Jr.; Hubbard, G. and Schlabritz-Loutsevitch, N. E. (2014): *Brucella papionis* sp. nov., isolated from baboons (*Papio* spp.). Int. J. Syst. Evol. Microbiol, 64 (12): 4120 - 4128. Doi: 10.1099/ijs.0.065482-0.

Yanagi, M. and Yamasato, K. (1993): Phylogenetic analysis of the family *Rhizobiaceae* and related bacteria by sequencing of 16S rRNA gene using PCR and DNA sequencer.FEMS Microbiol. Lett, 15: 115-120.

Yu, W. L. and Nielsen, K. (2010): Review of detection of *Brucella* spp. by polymerase chain reaction. Croat. Med. J., 51 (4): 306 -313.

Yue, X.; Schunter, A. and Hummon, A. B. (2015): Comparing multi-step IMAC and multi-step TiO2 methods for phosphopeptide enrichment. Anal. Chem., 87 (17): 8837 - 8844.

Zhou, W.; Merrick, B. A.; Khaledi, M. G. and Tomer, K. B. (2000): Detection and sequencing of phosphopeptides affinity bound to immobilized metal ion beads by matrix-assisted laser desorption/ionization mass spectrometry. J. Am. Soc. Mass Spectrom, 11 (4): 273 -282.

استطلاع جزيئي للبروسيلا بمطياف الكتلة المعتمد على زمن طيران الببتيدات المتأينة بالليزر الممتز بمساعدة وسط محيط

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الملخص العربي

البروسيلا هي عبارة عن جنس آخذ في الاتساع من مجموعة من مسببات الأمراض سالبة الجرام تعيش داخل الخلايا ولها مجال واسع من العوائل الحيوانية، وقد هدف هذا العمل إلى البحث في التعرف الجزيئي على البروسيلا بواسطة مطياف الكتلة المعتمد على زمن طيران الببتيدات المتأينة بالليزر الممتز بمساعدة وسط محيط، وذلك كبديل بروتيومي سريع لمعيار الذهب البكتيري، حيث أنشأت مكتبة لقمم منحنيات الكتلة الطيفية مؤلفة من 11 عترة بروسيلا مرجعية تتضمن أربعة أنواع لتغطية التعرف على الثلاثة أنواع من البروسيلا التقليدية المبلغ عنها في مصر، وقد تم رسم شجرة العلاقة المعتمدة على تطور النمط البروتيني وذلك لمطابقته بين سلالات البروسيلا المرجعية، واستنادًا إلى التصنيف الحيوي البكتيري والبروتيني لـعدد 45 معزولة حقلية، كشفت خريطة عن التوزيع الجغرافي للبروسيلا ميليتينسيز والبروسيلا أبورتس من 69 من المجترات غير المحصنة الإيجابية سيرولوجياً في 12 محافظة خلال عام 2015، وقد أعيد تقييم مطياف الكتلة المعتمد على زمن طيران الببتيدات المتأينة بالليزر الممتز بمساعدة وسط محيط كأداة جزيئية أحدثت ثورة في التعرف على البروسيلا مع استعراض إيجابيات وسلبيات هذه التقنية والإشارة إلى الأساليب الحديثة لمعالجة العقبات الحالية، وقد خلص الباحثون إلى أن النتائج البكتيريولوجية ونتائج مطياف الكتلة متطابقة تمامًا بفضل التنوع المحدود لمعزولات البروسيلا ومحدودية مكتبة قمم منحنيات الكتلة الطيفية، ولتعزيز الدقة نحو تمييز موثوق على مستوى أنواع البروسيلا، فإن تقنية مطياف الكتلة تستحق إثراءً انتقائياً للعينات، وقد تم اكتشاف كل من البروسيلا ميليتينسيز الطراز الحيوي 3 والبروسيلا أبورتس الطراز الحيوي 1 في الأبقار والجاموس، في حين أن البروسيلا ميليتينسيز الطراز الحيوي 3 فقط قد تم عزلها من المجترات الصغيرة وناقة ورجل.