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Bacteriological and molecular characterization of *Brucella* isolates from sheep and goats

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ABSTRACT

This study reported on a sporadic, naturally acquired infection of sheep and goats with *Brucella* on a private farm in El- Menofya Governorate, Egypt. The abortions, which occurred in a flock of 46 sheep and 33 goats, involved six ewes and five does at the third month of gestation. Serum samples from the flock were examined for *Brucella* antibodies using rose bengal test (RBT), Tube Agglutination Test (TAT) , indirect Enzyme Linked Immunosorbent Assay (I ELISA) and were further confirmed using complement fixation test (CFT) gave 18(39.1%), 16(34.8%), 19 (41.3%), and 17(37%) in sheep; respectively, while in goats revealed 11 (33.3%), 8(24.2%), 12(36.4%), and 11(33.3%) respectively. Tissue samples were collected from 28 positive animals, as detected by CFT, at slaughtering. The bacteriological results revealed 44 isolates which were biochemically identified as *B. melitensis* biovar3. The highest recovery rate was obtained from supra mammary lymph nodes (22/28; 78.6%), followed by spleen (15/28; 53.6%), and finally liver (7/28; 25%).The distribution of the virulence genes among 44 *B. melitensis* isolates revealed that Omp 25 recorded the highest incidence 44(100%), then followed wbkA 43(97.7%) and manB 42 (95.5%) . The high prevalence of virulence-associated genes among the *B. melitensis* isolates detected from different animal species in Egypt indicates a potential virulence of this bacterium. The authors concluded that the most frequent virulence genes are wbkA, manB and omp25 among isolates which are assumed to play a worthy function in the pathogenesis of brucellosis in this region.

INTRODUCTION

Brucellosis is an important zoonotic disease that causes huge economic losses to the livestock owners and is of great public health significance. It is a chronic infectious disease of livestock, rodents, marine animals and human being and is caused by facultative intracellular coccobacilli of genus *Brucella* **(Kavi et al. 2015),** Although brucellosis in livestock and human has been decreased through the prevention programs in many parts of the world, and it has been eradicated from several countries of the world, however it remains an uncontrolled problem in many regions especially of high

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endemicity such as the Mediterranean, Middle East, Africa, Latin America and parts of Asia **(DíazAparicio et al. 2013).**

Brucellosis is a disease of the sexually mature animals with preference of placenta, fetal fluids and tests of male animals. Brucellosis has been known as a global problem of wild and domestic animals, especially cattle, sheep and goats (**Wogayehu et al. 2020**).

Brucella melitensis biovar3 is the most common and predominant strain isolated from different animal species from almost all Egyptian Governorates **(Abdel- Hamid et al. 2016 Abdel Hamid et al. 2020)**.

Also **(Hegazy et al. 2022)** found that *B. melitensis* biovar3 was the predominant strain isolated from the typical (small ruminants) and atypical hosts (large ruminants) in Egypt. This finding indicates the potential cross species transmission of *B. melitensis* biovar3 from the original hosts to large ruminant species in the country, and this may be attributed to the uncontrolled movement of animals in infected areas, as well as the type of animal husbandry practiced **(Wareth et al. 2020).**

Brucella melitensis, includes three biovars (biovars 1, 2 and 3). All three biovars give rise to a disease in sheep and goats, and is highly pathogenic for humans, causing one of the most serious zoonosis in the world but their geographic distribution differs **(Rossetti et al. 2017).**

Although *Brucella abortus* and *Brucella suis* infections occur occasionally in small ruminants, but the clinical disease sounds to be scarce **(Pal et al. 2017).** Brucellosis may give rise to significant economic losses. In livestock, brucellosis results in decreased productivity, abortions and weak progeny and is a major barrier for commerce and export **(Godfroid 2017)** Regarding to human brucellosis is a severely debilitating disease that requires prolonged treatment, compliance of the patient, and results in considerable medical expenses in addition to loss of income due to loss of working hours **(Mohamed et al. 2018).**

When brucellosis is detected in a herd, flock, region, or country, international veterinary regulations impose restrictions on animal movements and trade, which result in huge economic losses. The economic losses as well as its zoonotic importance are the reasons why programs to control or eradicate brucellosis in cattle, small ruminants and pigs have been implemented worldwide **(Kandeel et al. 2014).**

Currently, diagnosis of this zoonosis is based on microbiological and serological laboratory tests **(Navarro et al. 2002).** Nucleic acid amplification methods such as PCR can overcome the limitation of conventional detection methods as they are rapid, sensitive, high specific and of low cost **(Sintayehu et al. 2015).**

Genetic and immunological evidence indicates that all members of the genus *Brucella* are closely related. Nevertheless, it has many virulence factors causing sever pathogenicity **(Gandara et al. 2001).** Differences in virulence have been observed in members of the genus *Brucella*, and the levels of virulence order shown in guinea-pigs seems to be similar to that in humans whereas, *B. melitensis* scored the high level of virulence followed by *B. suis* and *B. abortus* **(Smith and Ficht, 1990).**

Brucella employs a number of mechanisms for avoiding bactericidal responses inside macrophages. Unlike rough strains, smooth *Brucella* organisms engulfed by macrophages, proved to play a role in suppressing macrophage apoptosis by preventing lysosome phagosome fusion subsequently they have the ability to survive for longer periods inside macrophages **(Pei et al. 2006).**

Brucella spp. carry various furtive strategies to enter into host cells then propagate and overcome the host defense mechanism **(Martirosyan et al. 2011)**. A recent discovery of new a typical *Brucella* spp., with new genetic properties was recorded **(Zilberman et al. 2022)**. Therefore, it was expected that a new outbreak of brucellosis may occur in the future. *Brucella* was already exhibiting virulence factors required to form the infection due to their activation by erythritol **(Petersen et al. 2013).**

The constant researches for *Brucella* virulence genes such as cell envelope associated genes and other virulent genes are necessary to understand their role in *Brucella* pathogenesis, characterize the *Brucella* spp., genome and have efficient control measures **(Awwad et al. 2015).** As a result of the brucellosis' endemic status in Egypt and the need to develop new preventive measures against brucellosis, the aim of the current work is serological, bacteriological and molecular investigation of brucellosis among abortion storm in sheep and goat herds in private farm and animals in contact in such farm for the presence of brucellosis. Also, to study the presence and distribution of some virulence-associated genes in different *Brucella* strains among examined animals. The outcomes of this study are needed to highlight the role of virulence genes on the contagiousness of brucellosis and to aid in developing a vaccine candidate originating from local field strains to immunize native farm animals for the control of animal brucellosis and consequently, to minimize public health hazard.

MATERIALS AND METHODS:

Description of the farm and animal husbandry:

The farm involved in this study is private farm located in El- Menofya Governorate, Egypt.

The sheep flock Egyptian sheep(*Ovisori entalisaries*) and Baladi does (*Capra hircus*)**.** The flock consists of a total of 46 Egyptian sheep (*Ovisorientalisaries*). The population structure is consisted of 12 male (4 adult and 8 sub adults) and 34 ewes (6 adult aborted, 16 adult in contact and 12 sub adults).

The goat flock

The flock consists of 33 Baladi goats (*Capra hircus*). The population structure is consisted of 8 male (3 adult and 5 sub adults) and 25 does (5 adult aborted, 11 contact adult ewes and 9 sub adults).

The animals were maintained under a semi-intensive husbandry system, fed mainly on concentrates, but obtaining part of their roughage by grazing on open grassland on the farm. The females were mated by the males in the flock. The long-term plan was to increase the size of the flock, with adult rams being sold for meat during local festivals, and loaned to peasant farmers to upgrade the stocks of local sheep. In late January 2021, an outbreak of abortion involving 4 ewes and 2 does in the flock was reported. This was followed two months later by another incidence of abortion involving two ewes and three does. In each case the abortions occurred as short, sharp 'storms' at the third month of gestation. The animals had no history of vaccination.

Serum samples: Ten mL of blood was collected from jugular vein of examined sheep and goats. Collected samples were kept in a refrigerator overnight for serum separation then were centrifuged at 3000 rpm for 5 min. Clear sera were siphoned off and stored in cryotubes at -20C until its use for serological studies.

Tissue samples: From all serologically positive animals, tissue samples were taken from the lymph node, liver and spleen of seropositive sheep and goats. Then collected samples were transferred immediately to the laboratory for further bacteriological examination.

Serological assessments:

Rose Bengal test (RBT): All collected sheep and goats samples were tested using antigen stained with rose Bengal and buffered to a low pH, 3.65 ± 0.05 The (RBT) antigen (3 % cells) for small ruminants was prepared, standardized and verified in the Department of Brucellosis Research, Animal Health Research Institute, Dokki, Giza, Egypt according to the American method **(Alton et al. 1988)**. Any degree of agglutination was considered positive results. The serum samples and antigen were carried at room temperature (22°C ± 4°C).

2- Tube agglutination tests (TAT): All tested animal serum samples were examined by TAT using *B. abortus* concentrated antigen (white antigen). A visible agglutination at dilution of 1/40++ or more was considered positive **(Alton et al. 1988; MacMillan, 1990).**

Antigens for the RBT 8 and the white antigen were obtained from VSVRI (Abbasia Laboratories, Abbasia, Cairo, Egypt).

3-ELISA : All samples were analyzed also by *Brucella* i-ELISA kit that was performed

following manufacturer's instructions "(ID. vet, ID screen, Brucellosis serum indirect multispecies rue Louis Pasteur-Grabels – France).

4- All samples were further analyzed by CFT for confirmation of *Brucella* infection. The complement fixation test (CFT), standard *Brucella abortus* antigen , haemolysin , complement and control sera were obtained from NVSL/DBL, USDA, USA.

Phenotypical identification of *Brucella* species

Tissue samples (Lymph node, liver and spleen) of aborted sheep and goats positive on the CFT were processed aseptically by removing extraneous material and chopped into small pieces, and macerated using a 'stomacher' or tissue grinder with a small amount of sterile phosphate buffered saline (PBS). Then, the samples were inoculated onto *Brucella* Selective Agar with antibiotic supplement (Oxoid, Basingstoke, UK) and incubated at 37 °C both in the absence and presence of 5–10% CO2 and cultured plates were examined for *Brucella* spp. growth on day 4 and daily for 2 weeks. *Brucella*-suspected colonies characterized by typical round, glistening, pinpoint and honey drop-like appearance. Finally, the presumptive isolates were checked further by Modified Ziehl-Neelsen (MZN) staining, CO2 requirement and biochemical tests including catalase, oxidase, urea hydrolysis, nitrate reduction, H_2S production and growth on thionin and basic fuchsin dyes incorporated into trypticase soy agar at different concentrations, lysis by Tbilisi phage and agglutination (with A and M antisera) were done (**Quinn et al. 2004** and **Geresu et al. 2016).**

DNA extraction

DNA extraction from bacterial culture was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit. The DNA concentration was determined with spectrophotometer**.**

Oligonucleotide Primers.

Primers used were supplied from Metabion (Germany) and are listed in table (1). PCR amplification Primers were utilized in a 25µl reaction containing 12.5µl of Emerald-Amp Max PCR Master Mix ((Takara) Code No. RR310A kit Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5µl of DNA template. The reaction was performed in an Applied Bio-system (ABI) 2720 thermal cycler.

Analysis of the PCR Products

The products of uniplex PCR were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20µl of the products were loaded in each gel slot. A gel pilot 100 bp plus DNA ladder (Qiagen, Gmbh, Germany), gene ruler 100 bp ladder (Fermentas, Germany) and DNA ladder H3 RTU (Genedirex, Taiwan) were used to determine the fragment sizes. The amplified products in agarose gel were visualized by ultraviolet transilluminator after gel staining with ethidium bromide stain. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra). Sterile DNA-free water used as a control negative and *B. melitensis* biovar 3 reference strain (ATCC No., 23458) was used as control positive. Internal quality control samples were employed in the PCR process to ensure and exclude DNA contamination

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions for conventional PCR

Target gene	Sequence (5`-3`)	Amplified product (bp)	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension	Reference
wbkA	AATGACTTCCG CTGCCATAG ATGAGCGAGG ACATGAGCTT	931	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 50 sec.	35	72˚C 10 min.	**Awwad *et al*., 2015**
manB	GGCTGGTTCGA GAATATCCA CAATCGCATAC CCTGGTCTT	228	94˚C 5 min.	94˚C 30 sec.	58˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.	**Naseri *et al*., 2016**
Omp25	TTT CCG TGT CCA ATT ATG CTA ACCGCGCAAA ACGTAATTT	701	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.	**Awwad *et al*., 2015**

RESULTS

Table 2: Results of serological tests for the recognition of brucellosis in examined sheep

Sex	Animal status	Number examined	Serological tests							
			RBT		TAT		iELISA		CFT	
			NO	%	NO	%	NO	%	NO	%
Male (N= 12)	Adults	4	0	0	0	0	0	0	0	0
	Sub adults	8	0	0	0	0	0	0	0	0
	Total	12	0	0	0	0	0	0	0	0
Femals (N= 34)	Adults aborted	6	6	100	6	100	6	100	6	100
	Adults in contact	16	12	75	10	62.5	13	81.3	11	68.8
	Sub adults	12	0	0	0	0	0	0	0	0
	Total	34	18	52.8	16	47.1	19	55.9	17	50
Total (N= 46)		46	18	39.1	16	34.8	19	41.3	17	37

Table 3: Results of serological tests for the recognition of brucellosis in examined goats.

Sex	Animal status	Number examined	Serological tests							
			RBT		TAT		iELISA		CFT	
			NO	%	NO	%	NO	%	NO	%
Male (N= 8)	Adults	3	0	0	0	0	0	0	0	0
	Sub adults	5	0	0	0	0	0	0	0	0
	Total	8	0	0	0	0	0	0	0	0
Femals(N= 25)	Adults aborted	5	5	100	5	100	5	100	5	100
	Adults in contact	11	6	54.5	5	45.5	7	63.6	6	54.5
	Sub adults	9	0	0	0	0	0	0	0	0
	Total	25	11	44	8	32	12	48	11	44
Total (N= 33)		33	11	33.3	8	24.2	12	36.4	11	33.3

Table (4): Number of *Brucella* strains isolated from lymph nodes, spleen and liver sample, from serologically positive examined sheep and goats .

	Serologically positive animals	**L.N**		**Spleen**		**Liver**		**Total**	
		NO	%	NO	%	NO	%	NO	%
Sheep	Adults aborted **(N= 6)**	5	83.3*	4	66.7	2	40	11	25**
	Adults in contact **(N=** 11**)**	9	81.8	6	54.5	3	27.3	18	40.9
Goats	Adults aborted **(N=** 5**)**	4	80	2	40	2	40	8	18.2
	Adults in contact **(N=** 6**)**	4	66.7	3	50	0	0	7	15.9
Total	28	22	78.6	15	53.6	7	25	44	100

Serologically positive= CFT positive
*= percent calculated according to number of examined animals
**= percent calculated according to total number of isolated *Brucella* strains

Table (5): Prevalence of the virulence genes among 44 *B. melitensis* isolates according to animal species and status

Examined animals	**serologically positive animals**	**Organs**	**NO**	**Examined genes**		
				WbkA	**manB**	**Omp25**
				%	**NO**	**NO**
Sheep	Adults aborted **(N= 6)**	L.N	5	5	5	5
		Spleen	4	4	4	4
		Liver	2	2	2	2
	Adults in contact **(N=** 11**)**	L.N	9	9	9	9
		Spleen	6	6	5	6
		Liver	3	2	3	3
Goats	Adults aborted **(N=** 5**)**	L.N	4	4	4	4
		Spleen	2	2	2	2
		Liver	2	2	2	2
	Adults in contact **(N= 6)**	L.N	4	4	4	4
		Spleen	3	3	2	3
		Liver	0	0	0	0
Total			44	43	42	44
Percent				97.7%*	95.5%	100%

*=percent calculated according to total number of isolated *Brucella* strains

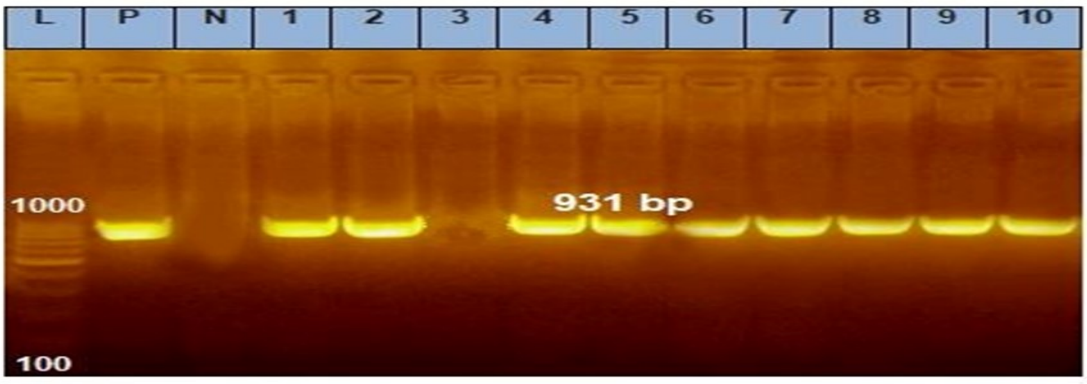

Fig 1. Agarose gel electrophoresis image of virulence factor gene wbkA in *B. melitensis* isolates, where L; Marker (100bp), P; positive control, N; Negative control. All samples shown positive PCR product for the wbkA virulence gene except samples numbers 3 was negative

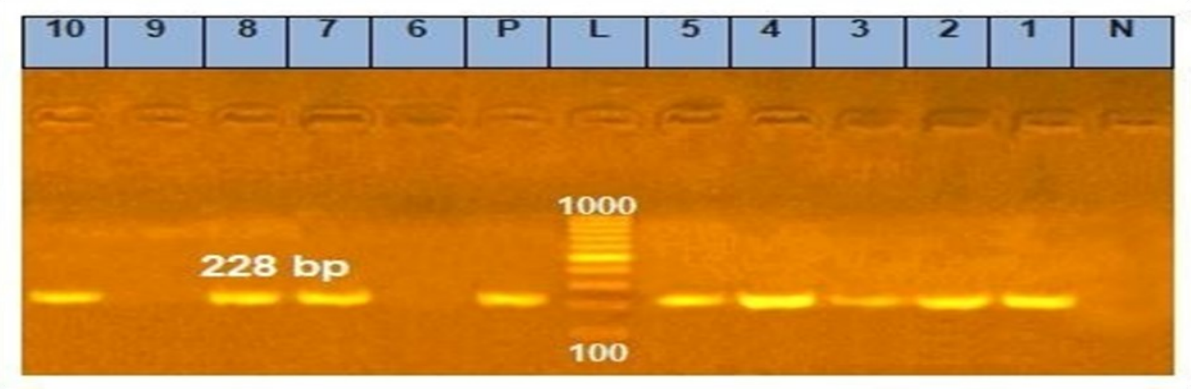

Fig 2. Agarose gel electrophoresis image of virulence factor gene manB in *B. melitensis* isolates, where L; Marker (100bp), P; positive control, N; Negative control. All samples shown positive PCR product for the manB virulence gene except samples numbers 6 and 9 were negative.

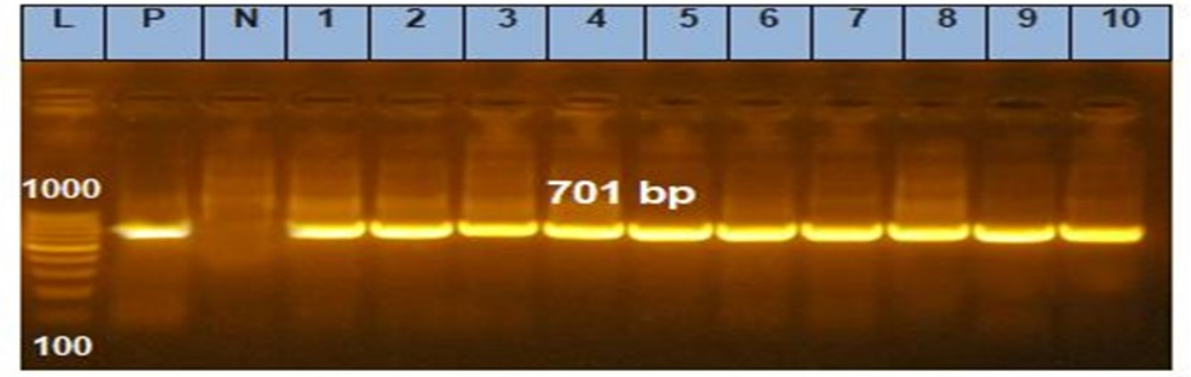

Fig. 3. Agarose gel electrophoresis image of virulence factor gene Omp25 in *B. melitensis* isolates, where L; Marker (100bp), P; positive control, N; Negative control. All samples shown positive PCR product for the Omp 25 virulence gene.

DISCUSSION

Brucellosis is an important zoonosis that causes abortion in naturally infected small ruminants and is of great public health concern in many countries **(Sadhu et al. 2015)**. *B. melitensis*is is the main etiologic agent of brucellosis in small ruminants. Ewes' and nanny-goats aborted fetuses and products derived from sheep and goats remain the main source of infections. **(Wogayehu et al. 2020).**

Many factors that affect brucellosis seroprevalence in small ruminants could be associated with frequent introduction of purchased animals into flocks, including the absence of quarantine/segregation, mixing of different species of infected flocks, improper safe hygienic disposal of aborted fetuses placental membranes, contact of healthy animals with contaminated drinking water, grassing yards and feed, and lack of vaccination and control strategies for small ruminants **(Unver et al. 2006).**

The presumptive diagnosis provided by the serological tests, is usually accepted as indication of brucellosis. Rose Bengal Plate Test (RBPT), Tube agglutination test agglutination Test (TAT), indirect ELISA (iELISA) and Complement Fixation Test (CFT), are utilized in this study for the detection of antibodies specific to *Brucella* spp**.** Serological examination performed by RB test in the present study gave higher number of positive samples 18 (39.1%) and 11 (33.3%) in examined sheep and goat respectively as RBT assay can detect antibodies of classes IgG1 and IgM against surface antigen lipopolysaccharides (LPS) of

smooth *Brucella* **(Davies, 1971).** These results indicate that RB test is not confirmative test for diagnosis of brucellosis. Although RB test is known to have many false positive or negative results, but generally it is simple, rapid and can be used as screening method for infection ***(*Hosein et al. 2017*).***

TAT assay is approved by the veterinary authority organization in Egypt. However, chronic carriers produce mainly IgG1that block the agglutinating activities of IgG2 **(Farina, 1985)** which may result in lower detection rates which was 16 (34.8%) and 8 (24.4%) in examined sheep and goat respectively. This may explain the lower number of positive samples detected by TAT in comparison to other serological tests used, excess of antibodies resulting in false negative reaction due to prozone effect **(Afify et al. 2013)**.

In the present study, iELISA provided positive reactors 19 (41.3%) in sheep and 12 (36.4%) in goat. Similar findings given by **(Saravi et al. 1995), (Hermoon et al. 2001)** who reported that iELISA has been evaluated for many years for their better sensitivity to detect anti-*Brucella* antibodies in all species especially small ruminant. Several studies reported that iELISA is more sensitive than conventional tests **(ElTahir et al. 2018 and Radulescu et al. 2007)**

The higher sensitivity of iELISA due to its recognition of cytosolic antigen S-LPS fragments may decrease cross-reaction with other Gram-negative bacteria share similar epitopes with *Brucella* **[OIE. (2018).** The present iELISA performance is consistent with the study of (**Nielsen et al. 2004).**

In the current study, the prevalence rates of brucellosis using CFT were 17 (37.0%) and 11 (33.3%) in sheep and goats respectively which nearly similar to results of RBT. These results coincided with (**Sintayehu et al. 2015)** who demonstrated that RBT and CFT were effective methods for the detection of *Brucella spp*. antibodies.

CFT is considered as gold standard serological test used for detection of brucellosis as it detect mainly IgG1 specific for *Brucella* infection and some IgM, but not IgG2 or IgA **(MacMillan 1990).**

(Stemshorn et al. 1985) reported that CFT is confirmatory test for the diagnosis of brucellosis due to the good balance of the sensitivity and specificity of the CFT was attributed to its high ability to detect low concentration of IgG1 characteristic of *Brucella* infection**.**

Trials for the isolation of the causative agents were carried out on 28 serologically positive animals. The highest recovery rate was obtained from supra mammary lymph nodes (22/28; 78.6%), followed by spleen (15/28; 53.6%), and finally liver (7/28; 25%) as described in Table 4. These findings come in accordance with **Aman et al. (2020)**. On the other hand, a higher rate of isolation of *Brucella* organism reported by **Khalafallah et al. (2020)** as culturing of tissue samples from lymph nodes, spleen and liver were 61.54%, 40.38% and 36.54% respectively

A total of 44 isolates of *Brucella* spp. were identified; all isolates were *B. melitensis*. Isolation of *Brucella* spp. confirmed active brucellosis in the animals tested. The low isolation rate of *B. melitensis* obtained in the present study from sero positive animals with a history of abortion was in agreement with **Çelebi and Otlu (2011) and Seleem et al. (2010)** Who reported that this low isolation rate might be because of the slow growing and fastidious nature of the pathogen. Furthermore, its isolation requires a large number of viable bacteria in clinical samples, proper storage and quick delivery to the diagnostic laboratory

The current study demonstrated 44 *Brucella* isolates which were identified as *B. melitensis* biotype3. This was supported previously by studies declared that brucellosis is endemic in Egypt particularly in the Nile Delta region and *B. melitensis* remains the more virulent strain with public health **(Wareth et al. 2014 and Ramadan et al. 2016).**

In the current study, DNA was successfully extracted from all 44 *B. melitensis* isolates obtained from sheep and goats. As expected the

wbkA, manB and omp25, genes assays with PCR produced amplicons of 228, 931 and 701bp respectively (Fig. 1, 2 and 3). Of the 44 *B. melitensis* strains; 43 (97.7%) isolates were positive for wbkA gene, 42 (95.5%) isolates carried manB gene and in 44 (100%) isolates omp25 gene was detected (Table 5). It is noteworthy to find that irrespective of the animal species from which *B. melitensis* was isolated, the distribution of virulence genes among the isolates was not affected by crossing the animal species host barrier. The same levels of distribution of the three virulence genes was observed in all *B. melitensis* isolates, under test, regardless of the animal species.

However, other researchers detected omp25 in 100% of 80 *B. melitensis* strains isolated from sheep and goats in Palestine, while the wbkA and manB genes were detected in 95% of the isolated strains **(Awaad et al. 2015).** These results nearly similar to the results obtained from *B. melitensis* in the current study indicating that the virulence genes were more predominant not only in *B. melitensis* strains isolated from Egypt but also in the other *B. melitensis* strains isolated from the Mediterranean Region.

At present, *Brucella* LPS encodes 32 virulence factors (**Pelerito et al. 2021**). The wbkA and manB genes play a role in intracellular survival and intracellular modulatory activity of Brucellae in host cells, besides; they are proved to protect the organisms from the host's defense mechanism **(Lapaque et al. 2005).**

LPS is the most significant virulence factor for *Brucella* which allowed to survive inside macrophages and other cells of the reticuloendothelial system by incidence of the O-side chain on the lipopolysaccharide of smooth strains. Those results was agreed with present study when, manB; wbkA was found in all *Brucella* isolates **(Caron et al. 1996; Lory and Tai, 1984).** Those was found a smooth strain is more virulent than rough strains

The wbkA gene encodes mannosyltransferase and manB gene encodes phosphorusmannomutase, both being involved in the LPS synthesis in brucellae. The smooth brucellae can escape the immune defense mechanism of the host by avoiding factors released from dead cells during apoptosis**.** The presence of such genes in the *Brucella* genome indicates their virulence. The obtained results in this study were in harmony with results obtained by other workers who found that wbkA and manB were found in all smooth *Brucella* isolates **(Caron et al. 1996).** This finding may be ascribed to the concept that smooth LPS of *Brucella* have many atypical features, relatively low toxicity for macrophages. The obtained results were in accordance with this concept, as *Brucella* isolates obtained in this study were isolated from aborted animals in and in contact animals with a known history of brucellosis. Our results are in agreement with another study that detected wbkA and manB in 8 *B. melitensis* isolates obtained from human patients in Babylon Hospital, Iraq **(Razzaq et al. 2014).**

Concerning to the critical role of manB gene among *Brucella* spp., many reports confirmed the contribution of manB genes in lipopolysaccharide synthesis which allowed the intracellular survival and protection against host defense (**Lapaque et al. 2005).**

These virulence determinants were vital for *Brucella* spp., to live, adapt intracellularly to inappropriate conditions and resist body immune response (**Saeedzadeh et al. 2013**). From these substantial genes, Omp25 was described as major surface proteins strongly contributed to the virulence of *Brucella* (**Martin et al. 2008).** Moreover, the major function of omp25 depends on suppression of the tumor necrosis factor alpha (TNF") produced by macrophages (**Jubier-Maurin et al. 2001).**

The outer membrane contains only two components that have been identified virulence factors: the lipopolysaccharide (LPS) and the outer membrane proteins (OMPs) **(Lory and Tai, 1984).** The serum of susceptible animals contains a globulin and lipoproteins that suppresses growth of nonsmooth, avirulant types and favor the growth of virulent types. Resistant animal species lack these factors, so that rapid mutations to avirulence can occur. **(Brooks et al. 2010).** There are important for explaining the differences in virulence and host

specificity of *Brucella* spp. **(Ratushna et al. 2005),** at the same time as **(Halling et al. 2006)** mentioned that because of the similarity among the genomic sequences of Brucellae spp. differences among them with regards to host favorite virulence and infections cycle could be due to subtle variations in the conserved DNA and differential expression of conserved genes, rather than due to sole genomic DNA fragments of genus *Brucella* the two chromosomes of *Brucella* differ in two significant properties. The source of replication of the large chromosome (ch I) is typical of bacterial chromosomes, while that of the small chromosome (ch II) is plasmid like mainly of the essential genes are located on chr I.

Omp25 from *Brucella* spp. is tightly associated with LPS, and so it is possible that such an interaction specifically impairs the *Brucella* LPS signaling leading to TNF-α production while not affecting the messages linked to IL-1β, IL-6, and/or IL-8 production **(Martirosyan et al. 2011).**

Finally, due to the seriousness of ovine and caprine brucellosis and its impact on public health, further studies are needed to spot highlights on the role of these genes and others in the contagiousness of brucellosis and the ability to produce types of vaccines to control or minimize the disease incidence.

CONCLUSSION

In the present study *B. melitensis* biotype3 was isolated from seropositive sheep and goats with history of recent abortion and in contact animals. The organisms were isolated from lymph nodes, spleen and liver of examined animals. Hence, it is of practical importance to isolate *Brucella* spp. to design and utilize effective *Brucella* vaccines in Egypt.
The most frequent virulence genes are wbkA, manB and omp25 among isolates which are assumed to play a worthy function in the pathogenesis of brucellosis in this region. Moreover, it may be helpful for authorized affairs to develop a strategic plan for the prevention and eradication of this disease. The potential risk of these biohazard virulent strains reflects the contagiousness of the disease in animals in Egypt and constitutes a real threat to public health.

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