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# ISOLATION, IDENTIFICATION, AND MOLECULAR CHARACTERIZATION OF *BRUCELLA MELITENSIS* FROM RETROPHARYNGEAL LYMPH NODE OF SHEEP IN ALMONOFIYAH, EGYPT

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## Abstract

Brucellosis is economically important infection in Egypt, and the brucellosis causative agent has not been genetically characterized in detail. In this study *Brucella melitensis* bv.3 was isolated and characterized from retropharyngeal lymph node and other lymphoid organs of sheep and goats from Almonofiyah Governorate & Albehera Governorate, Egypt, and to characterize one of the *Br. melitensis* isolates by 16S rRNA gene sequencing and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR_RFLP) of outer membrane protein (*omp2a*) gene. *Br. melitensis* was isolated and identified from 17 out of 47 (36.2%) samples from lymphoid organs of sheep& goats. The 16S rRNA gene of one of the *Br. melitensis* isolate was sequenced and identical as *Br. melitensis* bv.3 strain (Gene Bank accession No. KF177277.1). The genetic characterization of *Br. melitensis* in this study can be used for epidemiological studies, control of ovine brucellosis. In our knowhow this is the first report of the16S rRNA gene sequence of *Brucella* in Egypt.

**Key words**: *Brucella melitensis*, 16S rRNA gene, Outer membrane protein (*omp2a*) gene.
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## Introduction

Brucellosis caused by gram-negative coccobacilli of the genus *Brucella* which infect many of cattle, sheep, goats, and other livestock (Corbel, 1997). It has been recognized as a global problem of wild and domestic animals, especially cattle, sheep and goats (Rijpens *et al*., 1996).

Since the discovery of *Brucella melitensis* by David Bruce in 1887, several species have been identified, such as *Br. abortus* (which infects cattle), *Br. melitensis* (which infects sheep and goats), *Br. suis, Br. neotomae, Br. ovis*, and *Br. canis* (El-Bayoumy, 1989). Although brucellosis has been controlled in most industrialized countries, it remains a major problem in the Mediterranean region, western Asia, Africa, and Latin America (Pappas *et al*., 2006). It can cause appreciable economic losses in the livestock industry because of abortions, decreased milk production, sterility, and veterinary care and treatment costs (Corbel, 1997). Brucellosis was first reported in Egypt in 1939 (Refai, 2002).

Control programs for brucellosis in Egypt have used 2 methods: vaccination of all animals and slaughter of infected animals with positive serologic results.

The difficulty of accurately detecting all infected animals, especially carriers, is a major limitation of these programs. To enhance efficiency of brucellosis-specific prophylaxis, early detection of brucellosis by highly sensitive and specific methods is needed. Egypt has mixed populations of sheep, goats, cattle, and buffaloes. The number of buffaloes in Egypt is higher than in any other country in the Near East region (Cloeckaert *et al*., 1995). In addition to high prevalence rates of *Br. melitensis* infections in sheep and goats, *Br. melitensis* infections of cattle and buffaloes have increased in Egypt (Pappas *et al*., 2006).

Since the genomes of *Brucella* spp. are highly homogeneous (>95 % homology at the DNA-DNA pairing) among each other (Gee *et al*., 2004), and 16S rRNA genes have very little or no polymorphism among the isolates and biovars, several researchers proposed that all *Brucella* spp. should be placed in one specie name (Vizcaino *et al*., 2000).

**Aims of the work**

This study aimed to isolate and characterize of *Brucella melitensis* from retropharyngeal lymph node and other lymphoid organs of sheep and goats from Almonofiyah Governorate & Albehera Governorate, Egypt, and to characterize one of the *Br. melitensis* isolates by 16S rRNA gene sequencing and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR_RFLP) of outer membrane protein (*omp2a*) gene.

**Materials and Methods**

**Serological Examination:**

All sera were screened for antibodies against *Brucella* by Buffered acidified plate antigen test (BAPT), and Rose Bengal plate test (RBPT) as field tests. All positive serum samples were further retested by SAT, Serum Agglutination Test, Riv.T: Rivanol Test, as qualitative confirmatory tests described by (Alton *et al*., 1988)**.**

**Tissue specimens:**

Different lymph nodes (retropharyngeal, prescapular, prefemoral, internal iliac and supramammary) and spleen were collected from carcases of all serologically positive animals in different Northern Governorate areas, Egypt.

**Selective medium for isolation of *Brucella spp.***

Bacto-*Brucella* agar, pH 7.0 ± 0.2 at 25 °C (catalogue no. DF0964-01-3, Difco Laboratories, Detroit, Mich., USA) was used with 5% v/v filter-sterilized heat-inactivated *Brucella*-seronegative bovine serum plus 20% dextrose. Combinations of Antibiotics of were added to 1 liter of melted and cooled (56 °C) agar (Ewalt *et al*., 2001).

**Media for biovariety identification of *Brucella* strain**

**Media for dye sensitivity of *Brucella* isolates**

Tryptic soy agar was used as a basal medium with different dyes, 0.1% stock solution of each dye was made in distilled water and subjected to flowing steam for 20 minutes then added to the molten agar while still hot. Stock solutions were renewed monthly.

1. Thionin medium (Alton *et al*., 1975)

   Thionin (Lauth's Violet; 3,7-diamino-5-phenothiazinium acetate, catalogue no. T 7029, Sigma Chemical Co., St. Louis, MO 63178, USA) was finally diluted 10 µg.ml$^{-1}$, 20 µg.ml$^{-1}$ and 40 µg.ml$^{-1}$ of medium.

2. Basic fuchsin medium (Alton *et al*., 1975)

   Basic fuchsin (Basic Red 9; Difco catalogue no. DF0191-13-4) was finally diluted to 10 µg.ml-1 and 20 µg.ml-1 of medium.

**Metabolic characteristics:-**

Oxidative metabolic studies were conducted by using substrate specific tetrazolum reduction (SSTR) test (Broughton and Jahans, 1997; Ewalt *et al*., 2001).

**Biotyping tests**

The $CO_2$ requirement, $H_2S$ production, growth in the presence of thionin (1: 25,000, 1:50,000, and 1:100,000 dilutions) and basic fuchsin (1:50,000, and 1:100,000 dilutions) dyes, and agglutination with monospecific A, M and R antisera, were performed as the methods of (Alton *et al*., 1988). *Brucella* monspecific antisera monospecific A, M and R *Brucella* antisera were offered by the NVSL, Ames, IA 50010, USA. For enough amounts, more sera were prepared.

**DNA isolation**

After 3 days of incubation, 4-5 colonies identified as *Brucella* sp. were taken with a loop and suspended in 1 ml of phosphate-buffered saline (PBS, 137 mM NaCl, 10 mM Na2HPO4, 2.7 mM KCl 1.8 mM KH2PO4 [pH 7.2]). After centrifugation, the bacterial pellet was resuspended with 100µl of PBS and the cells were lysed with the addition of 400 µl of NETS buffer (10mM NaCl, 1 mM EDTA, 10 mM Tris-Hcl [pH7.6], 0.5%SDS). After addition of 50 µg of Proteinase K (MBI Fermantes, St. Leon-Rot, Germany), the mixture was incubated at 65°C for 20 min. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and vortexed. The aqueous layer was carefully removed to a new tube and 0.1 volume of the 3M sodium acetate and 2-3 volumes of cold absolute ethanol were added. The mixture was incubated at -20°C for at least 2 hours. After centrifugation at 13,000 rpm for 10 min, supernatant was discarded and the pellet was washed with 70 % of ethanol. After final centrifugation, the pellet was air dried and resuspended with 40µl of nuclease-free distilled deionized sterile water (Unver *et al*., 2006).

Identification by PCR

The PCR was used to confirm identification of one *Brucella* sp. isolate from retropharyngeal lymph node of sheep in Almonofiyah, Egypt as described by Bricker and Halling (Bricker, and Halling, 1994) using two primers (16srRNAF1: 5' AGAGTTTGATCCTGGCTCAG 3' and 16srRNAR1: 5' AATCTTGCGACCGTAGTCCC 3' ) Table 1. Briefly, the amplification was carried out in a 50-µl reaction mixture including PCR buffer, 1.5 mM MgCl2, 20 pmol of primer pairs, 0.2 mM each of dNTP mixture, 1.5 U of Taq DNA polymerase and 5 µl of DNA, with 3 min of denaturation at 95 °C followed by 35 cycles each consisting of 1 min of denaturation at 95°C, 1.5 min of annealing at 56°C and 1.5 min of extension at 72°C. The final extension was allowed to continue for 7 min. DNA isolated from Rev.1 strain of *Br. melitensis* and PCR reaction mixtures without addition of template were used as positive and negative controls, respectively. PCR products were electrophoresed in 1.5% agarose gels and visualized with ethidium bromide (EtBr) cited in (Unver *et al*., 2006).

**Table 1: PCR primers used in this study (Unver *et al*., 2006)**

| Primer names | Sequences (5' -3') | Target gene |
| --- | --- | --- |
| 16SrRNAF1 | AGAGTTTGATCCTGGCTCAG | *16s rRNA* |
| 16SrRNAR1 | AATCTTGCGACCGTAGTCCC | *16s rRNA* |
| 2Aa | GGCTATTCAAAATTCTGGCG | *omp2a* |
| 2aB | ATCGATTCTCACGCTTTCGT | *omp2a* |

Sequence Analysis of 16S rRNA Gene

One of the *Br. melitensis* isolate was used to amplify and sequence nearly entire 16S rRNA genes for further genetic analyses. 16S rRNA genes from this sample was amplified with primer pairs 16SrRNF1 - 16SrRNR1, designed to amplify 414 bp fragments, Using the molecular biology resource facility at VACSERA (VACSERA, Cairo, Egypt), sequencing was performed using ABI PRISM dye terminator cycle sequencing kit with AmpliTaq DNA polymerase and an Applied Biosystems 377 DNA sequencer (Perkin-Elmer, Foster City, Calif.). The sequence was analyzed using the BLAST program (National Centre for Biotechnology Information) to determine the closest available database sequences. Selected sequences were aligned using the Clustal W program.

**Polymerase Chain Reaction (PCR)-Restriction fragment length polymorphism (RFLP) analyses of *Brucella* isolate**

For genotyping of one of the *Br. melitensis* isolate comparing with reference strains were obtained from the Veterinary Sera and Vaccine Research Institute (VSVRI), Abbassiya, Cairo 11517, Egypt. Outer membrane protein gene (*omp2a* gene) was amplified by PCR using two primers (2aA: 5' GGCTATTCAAAATTCTGGCG 3' and 2aB: 5' ATCGATTCTCACGCTTTCGT 3'). The primers (Table, 1) and amplification conditions were identical with that of described by Cloeckaert *et al*. (1995), five µl of isolated DNA was used as template for omp2a amplification and 10 µl of PCR product was digested with 1 U of PstI

restriction enzyme (Invitrogen Co., San Diegio, CA, USA) which can differentiate the 2 different restriction profiles derived from *B. melitensis* strains demonstrated by Cloeckaert *et al*., (1995). Restriction digestion was performed with buffer and incubation temperature as described by the manufacturer. The digested PCR product was electrophoresed in 1.5% agarose gels and visualized with Ethidium Bromide (EtBr).

**Ultra Structural Examination:**

Small tissue specimen was taken from retropharyngeal lymph nodes for ultra-structure examination. The specimens were fixed in 5% cold cocodylate buffer glutraldehyde (4°C 0.1N, pH 7.2) then kept at 4°C until processing for electron microscopic examination (Bancroft and Stenes, 1982), this work was done at the electron microscope unit, Assiut University, Egypt.

**Results and Discussions:-**

Serological examination for incidence of *Brucell*a spp. using field tests (BAPT; Buffered acidified antigen plate test, RBPT: Rose Bengal plate test, as qualitative confirmatory tests SAT: Serum Agglutination Test, Riv.T.: Rivanol test) revealed that 47 out of 65 serum samples were positive result, 7 samples from Alsharkiya Governorate and 11 samples from Alexandria governorate exhibited negative qualitative confirmatory tests.

*Brucellae melitensis* bv.3 was isolated from 17 out of 47 (36.2%) lymph node tissues from infected sheeps and goat's from Almonofiyah governorate and Albehera governorate, Egypt on Bacto-Brucella agar medium& Tryptic soy agar were used as a basal medium with different dyes and identified by oxidative metabolic reactions and biotyping tests. The distinction between biovars within *Br. melitensis* is somewhat subjective as the three biovars present identical phenotypes when grown on agar plates with fuchsine and thionin. They differed only by their agglutination in anti-A or anti-M monospecific sera (Table 2).

The microorganisms were isolated from retropharyngeal, prescapular, prefemoral, internal iliac, and supramammary content in all *Br. melitensis*-positive samples. The

*Brucella* spp. are the etiological agents of animal and human brucellosis. *Brucellae* are Gram-negative bacteria, stained red using the modified Ziehl Neelsen technique (Stamp *et al*., 1950) and appearing as coccoid or short rod shaped cells from 0.50.7x 0.6-1.5 microns in size.

**Table 2: Identification of *Br. melitensis* field isolated strain from different source at Biovar level.**

| *Br. melitensis* Field isolates | | | $CO_2$ Requirements | $H_2S$ production | Growth on days | | | | | Monospecific Antisera | | | Bio variety Metabolic pattern |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Source host | Total number | | | Thionin | | | Fuchsin | | A | M | R | |
| | | | | | a | b | c | a | b | | | | |
| *Br. melitensis* field isolates | sheep | 9 | -ve | -ve | -ve | +ve | +ve | +ve | +ve | +ve | +ve | -ve | *Br.melitensis* bv.3 |
| | goats | 8 | -ve | -ve | -ve | +ve | +ve | +ve | +ve | +ve | +ve | -ve | |
| Reference strains | *Br. melitensis16 M* | | -ve | -ve | +ve | +ve | +ve | +ve | +ve | -ve | +ve | -ve | 1 |
| | *Br. abortus 544* | | +ve | +ve | -ve | -ve | +ve | +ve | +ve | +ve | -ve | -ve | 1 |
| | *Br. suis133* | | -ve | -ve | -ve | +ve | +ve | -ve | -ve | +ve | -ve | -ve | 1 |

a: Dye concentration 1:25,000(40ug/ml) A: A monospecific antisera
b: Dye concentration 1:50,000(20ug/ml) M: M monospecific antisera
c: Dye concentration 1:100,000(10ug/ml) R: Rough *Brucella* antisera
-ve: Negative result +ve: Positive result

Identification of an atypical *Br. melitensis* biovar 1 phenotype in Israel (Banai *et al*., 1990) that resembled *Br. melitensis* vaccine strain Rev. 1 by its susceptibility to these dyes. As Rev. 1 vaccination is common in Israel and because adverse Rev. 1 like strains have been frequently isolated (Banai, 2002) a concern was raised regarding reversion of the vaccine strain to a virulent phenotype.

The studies in various parts of Egypt indicate that the *Br. melitensis* biovar 3 isolated from sheep, goats (El-Bayoumy, 1989; Sayour *et al*., 1970) and cattle (El-Gibaly, 1969; Montasser, 1991) Confirmatory diagnosis must be provided by the isolation of etiological agents. Therefore, the isolation of *Br. melitensis* is important to study the epidemiology of brucellosis. The isolation of 39 *Br. melitensis* strains from 106 (32 in cattle, 25 in sheep and 5 in goats) indicated very high prevalence of *Br. melitensis* infection among these animals in this region and due to that, the disease may threat human and animal health which was coincide (Esmaeil *et al*., 2008; Ewalt *et al*., 2001; Vizcaino *et al*., 2000).

**One of the *Br. melitensis* isolate was used for genetic characterization.**

For this purpose, partially 16S rRNA gene of this isolate was amplified and sequenced 414bp. The obtained 16S rRNA gene was aligned and compared with other Gene Bank-accessible 16S rRNA gene sequences of *Br. melitensis* and other *Brucellae* spp. The 16S rRNA sequence obtained in the current study was identical to that of the recent sequence of type strain of *Br. melitensis* strains (Gene Bank accession number **KF177277.1**).

```
1     cgggtgagta   acgcgtggga   acgtaccatt   tgctacggaa   taactcaggg   aaacttgtgc
61    taataccgta   tgtgcccttc   gggggaaaga   tttatcggca   aatgatcggc   ccgcgttgga
121   ttagctagtt   ggtggggtaa   aggctcacca   aggcgacgat   ccatagctgg   tctgagagga
181   tgatcagcca   cactgggact   gagacacggc   ccagactcct   acgggaggca   gcagtgggga
241   atattggaca   atgggcgcaa   gcctgatcca   gccatgccgc   gtgagtgatg   aaggccctag
301   ggttgtaaag   ctctttcacc   ggtgaagata   atgacggtaa   ccggagaaga   agccccggct
361   aacttcgtgc   cagcagccgc   ggtaatacga   aggggggcta    gcgttgtttc   ggat
```

**Fig. 1: 16S rRNA gene sequence.**

**Table 3: The similarity of *Brucella melitensis* strain Almonofiyah1 16S ribosomal RNA gene, (gb-KF177277.1) and other 16s rRNA gene sequences of *Br. melitensis* preset on data base.**

| Description | Similarity | Accession numbers |
|---|---|---|
| *Brucella melitensis* strain Almonofiyah1 16S ribosomal RNA gene, partial sequence | 100% | KF177277.1 |
| *Brucella melitensis* biovar *Abortus* 2308 strain 2308 16S ribosomal RNA, complete sequence | 99% | NR_074149.1 |
| *Brucella melitensis* bv. 1 str. 16M strain 16M 16S ribosomal RNA, complete sequence | 99% | NR_074111.1 |
| Brucella melitensis strain DRDEBM0902 16S ribosomal RNA gene, partial sequence | 99% | JF939172.1 |
| *Brucella melitensis* strain DRDEBM0903 16S ribosomal RNA gene, partial sequence | 99% | JF939173.1 |
| *Brucella melitensis* strain DRDEBM0908 16S ribosomal RNA gene, partial sequence | 99% | F939176.1 |
| *Brucella melitensis* M28 chromosome 2, complete sequence | 99% | CP002460.1 |
| *Brucella melitensis* M5-90 chromosome II, complete sequence | 99% | CP001852.1 |
| *Brucella melitensis* NI chromosome II, complete sequence | 99% | CP002932.1 |
| *Brucella melitensis* NI chromosome I, complete sequence | 99% | JF939176.1 |
| *Brucella melitensis* strain MY/2009/1483 16S ribosomal RNA gene, partial sequence | 99% | JN571438.1 |
| *Brucella melitensis* strain DRDEBM0904 16S ribosomal RNA gene, partial sequence | 99% | JF939174.1 |

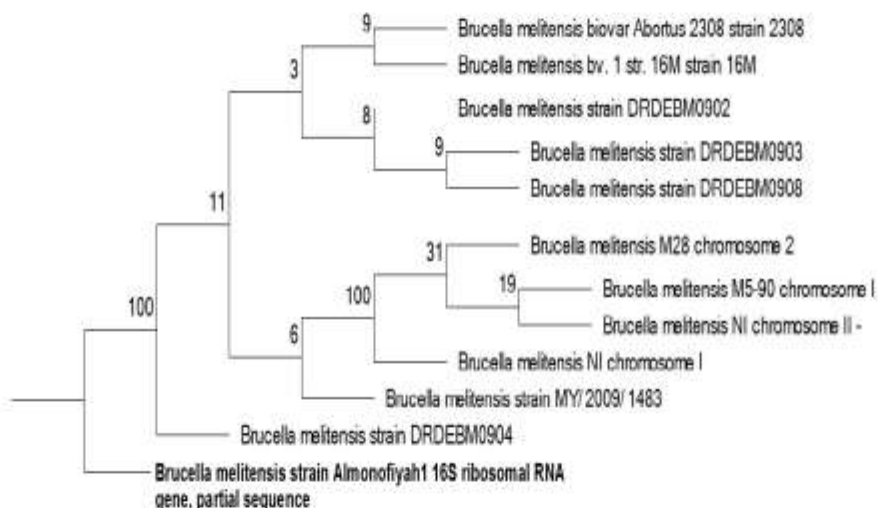


**Fig. 2: Phylogenetic tree for *Brucella melitensis* strain Almonofiyah1 16S ribosomal RNA gene, partial sequence (KF177277.1) compared with other 16s rRNA genes of *Br. melitensis* preset on data base.**

Analyses of 16S rRNA gene have been extensively used for molecular detection or taxonomic analyses of many different bacterial species (Gee *et al*., 2004)

New molecular methods, such as amplification of the insertion sequence (IS711) (Bricker and Halling, 1994) and restriction fragment length polymorphism analysis (RFLP) of the omp2a and omp2b genes (Broughton and Jahans, 1997), have been developed to identify *Brucella* strains at the genetic level.

**PCR-RFLP analyses**:

Omp2a gene was amplified from one of the isolate using 2aA and 2aB primers, about 1100 bp PCR product was observed on agarose gel (Fig.3). PstI was chosen for digestion of amplified omp2a since it has the only restriction site polymorphic between two groups of *Br. melitensis* strains (Cloeckaert *et al*., 1995).

The restriction digestion of the amplicon by PstI generated 600, and 450bp DNA fragments (Fig. 3) that similar with reference strain (*Br. melitensis* bv.3).

According to our data it has become clear to us that amplification and sequencing of 16S rRNA gene did not clarify beyond doubt that the *Br. melitensis* were at bv. 3, but PCR_RFLP provided an evidence look like finger print; our Isolates were *Br. melitensis* bv. 3.

According to this study the *Br. melitensis* bv.3 is the most pathogen of infected sheep and goats with Brucellosis among different *Brucellae* species in Northern areas, Egypt.

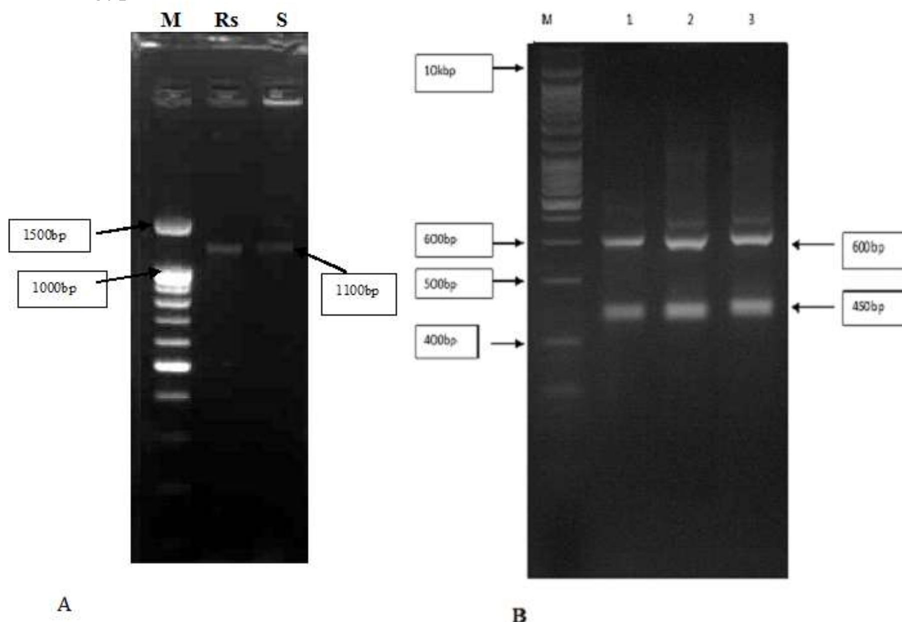


**Fig. 3: A: PCR product amplified from *omp2a* gene of one of the *Br. melitensis* isolate, B; Restriction pattern of PCR-amplified omp2a gene by PstI. Amplified products were resolved on agarose gels containing Et Br. Lanes M, molecular size markers; Lane RS, PCR product of *omp2a* gene of *Br. melitensis* bv.3 reference stain; S, PCR product of *omp2a* gene of *Br. melitensis* isolate; Lane 1, PCR-RFLP bands of *omp2a* gene of *Br. melitensis* isolate; Lanes. 2, 3 PCR-RFLP bands of *omp2a* gene of *Br. melitensis* bv.3 Ref. strains*;* Sizes of markers are indicated on the left.**

Several other gene targets were found to be more polymorphic than 16S rRNA gene among isolates to be used for genotyping of *Brucellae* (Moreno *et al*., 2002).

The omp2a and omp2b genes encoding 36 kDa OMPs of *Brucella* were reported to be highly diverse among *Brucella spp*., biovars and strains (Gee *et al*., 2004). Since then, this method was extensively used to genotype *Brucella* isolates (Bricker, and Halling, 1994).

PstI site polymorphism of its omp2 gene was previously used to differentiate *Br. melitensis* field isolates from that of vaccine (Rev.1) and prototype (16M) strains (Bardenstein, 2002). Rev.1 strain was found to be responsible for some of the brucellosis cases in humans and animals most likely derived from the adverse effects of the vaccination (Bardenstein *et al*., 2002).

**Electron Microscopical Finding**:

Electron microscopy revealed presence of moderate aggregations (clusters) of dark bodies of intact cocobacilli within the cytoplasm of macrophages of medullary sinuses of lymph node (Fig. 4).

Transmission electron microscope (TEM) studies of rough strain brucella infection showed that in addition to necrosis, brucella-infected macrophages underwent oncosis, which is a prelethal pathway leading to cell death characterized by cell organelle swelling, cell blebbing and increased membrane permeability (Majno and Joris, 1995; Fink and Cookson, 2005). These findings were confirmed by previous reports indicating that infected cells were not killed via apoptosis (Pei and Ficht, 2004).The outcomes of infection can be explained as the organism can reach replication niches and survive and the host cells will be killed. Otherwise the bacteria will be cleared by the host cells (Pei *et al.*, 2006).

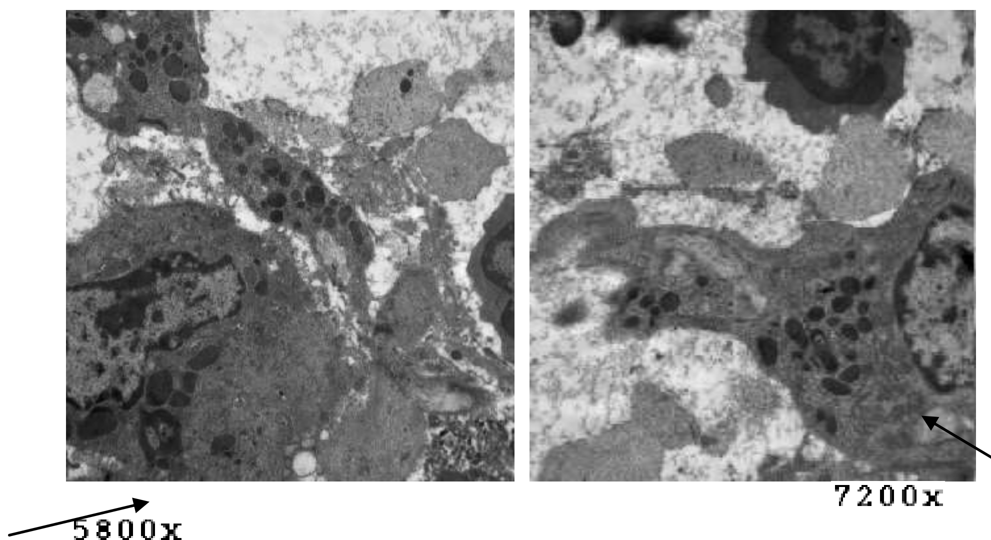


**Fig. 4: Electron micrograph revealed the presence of moderate aggregates of dark bodies of intact coco bacilli within lysosomes in cytoplasm of macrophage in the medullary sinuses of lymph node. (Uranyl acetate, X7200, X5800).**

**Conclusion**

Our data in the current study showed that 47 animal out of 65 were infected with *Br. melitensis* from 2 out of 4 northern Egyptian governorate performed by serology tests (Buffered acidified plate test, Rose Bengal plate test. Serum agglutination test, and Rivanol test). We isolated 17 bacterial isolates out of 47 lymph node samples, these bacteria identified as *Br. melitensis* bv.3 by biochemical tests. One of the *Br. melitensis* isolate was used for genetic characterization; 16S rRNA sequence obtained in the current study was identical to that of the recent sequence of type strain of *Br. melitensis* (Gene Bank accession number KF177277.1); at the PCR-RFLP analyses, PstI restriction enzyme was chosen for digestion of amplified *omp2a* gene that generated 600, and 450bp DNA fragments. *Br. melitensis* bv.3 is the most pathogen of infected sheep and goats with Brucellosis among different *Brucellae* species in Northern areas, Egypt.

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## الملخص العربى

## العزل والتعريف والتوصيف الجزيئى لمعزولة من بكتريا البروسيلا *ميلتينسيس* من الغدة الخلف بلعومية للاغنام فى المنوفية, مصر.

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ثم التعرف علي بروسيلا ملتينسيس للمرة الأولي في مالطا عام 1887م بواسطة العالم بروس وكانت أول التقارير المسجلة عن البروسيلا في إفريقيا كان في جنوب إفريقيا عام1906 م، تم اكتشاف البروسيلا في الحيوان في مصر سيرولوجياً للمرة الأولي عام1939 م. مرض الإجهاض المعدى (البروسيلا) هو مرض يصاب به الحيوان (مثل الماشية - الأغنام - الماعز - الخنازير - الكلاب - الجمال - الخيول)، وكذلك الإنسان وينتشر المرض فى جميع أنحاء العالم وعلى الأخص مناطق التربية. وكما نعلم أن نظام الزراعة فى مصر يقوم على الحيازة الصغيرة للأرض وحيازة ماشية واحدة أو أكثر للفلاح وهذا النظام يمثل غالبية تعداد الماشية فى مصر فهى معزولة بطبيعتها عن الماشية الأخرى ونسبة الإصابة بها صغيرة جداً, أما الحيازة الكبيرة للماشية، والتى تتمثل فى المزارع المتخصصة للحكومة والقطاع العام والخاص، فهى تمثل نسبة صغيرة من تعداد الماشية فى مصر، ونظراً لأن هذا المرض يعتبر مرض قطعان فتتركز الإصابة حالياً فى الحيازة الكبيرة للماشية.

أجريت هذة الدراسة على بعض الحالات المرضية للأغنام والماعز فى عدة محافظات فى شمال مصر وقد هدفت هذة الدراسة لعزل وتعريف بكتريا البروسيلا من عينات مرضية مختلفة بواسطة الاختبارات السيرولوجية والبيوكيميائية وكذلك تعريف معزولة من بكتريا البروسيلا والتى عزلت من العقدة اللمفاوية الخلف بلعومية بواسطة التوصيف الجزيئى باستخدام تقنية تفاعل البلمرة المتسلسل للحمض النووى ال د ن أ لجين 16S rRNA وكذلك تفاعل PCR-RFLP لجين البروتين الخارجى لغشاء الخلية omp2a gene , كذلك الفحص بواسطة الميكروسكوب الالكترونى لنسيج الغدة اللمفاوبية.

**وكانت النتائج كالتالى:**

1- تم فحص عدد 65 حيوان (35 من الأغنام و 30 من الماعز) فى محافظات المنوفية والبحيرة والشرقية والإسكندرية سيرولوجيا باستخدام اختباري الانتيجين المخمد المحمض الشريحي و الروزبنجال وكانت النتائج ايجابية.

وعند التأكيد علي نتائج الفحص الأولية بواسطة استخدام اختباري الانتيجين المخزن المحمض الشريحي و الروزبنجال بالإضافة إلي اختبارات التلزن الأنبوبي و الريفانول كانت النتائج إيجابية لعدد 47 حيوان فى محافظتى المنوفية والبحيرة وسلبية لعدد 7 حيوان فى محافظة الشرقية و11 حيوان فى محافظة الاسكندرية.

2- تم عزل 17 معزولة لبكتريا بروسيلا ميلتينسيس من أصل 47 عينة إيجابية من أنسجة الغدد اللمفاوية المختلفة للحيوانات المصابة وتم تعريفها بالاختبارات البيوكيميائية وكانت كلها بروسيلا ميلتينسيس من النوع 3 .

3- تم تعريف معزولة واحدة لبكتريا بروسيلا ميلتينسيس من النوع 3 عزلت من الغدة الليمفاوية الخلف بلعومية لأحدى الأغنام المصابة بواسطة إختبار تفاعل البلمرة المتسلسل لجين 16S rRNA وتم تسجيلة فى بنك الجينات الامريكى تحت رمز الموافقة KF177277.1, وكذلك باستخدام تقنية PCR-RFLP لجين البروتين الخارجى لغشاء الخلية omp2a gene ومقارنتة بعينة مرجعية تبين أن المعزولة لبكتريا بروسيلا ميلتينسيس من النوع 3 وذلك للمرة الاولى فى مصر.

4- الفحص الدقيق بالميكروسكوب الالكترونى لنسيج مصاب من الغدة اللمفاوية الخلف بلعومية لأحدى الأغنام أظهر تجمعات كثيف داكنة اللون من البكتريا مع الليسوسومات داخل خلايا الماكروفاج.