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## LIST OF ABBREVIATIONS

ABBREVIATIONS	TERM
<b>AHRI</b>	Animal health research institute
<b>AMOS</b>	Abortus, Melitensis, Ovis, Suis
<b>BAPT</b>	Buffered antigen plate test
<b><i>Bcsp31</i></b>	Brucella genus specific gene
<b><i>bvfA</i></b>	Brucella virulence factor A gene
<b>Cfu/g</b>	Colony forming unit per gram
<b>CT</b>	Cycle threshold
<b>dNTPs</b>	De oxy nucleotide tri phosphates
<b>EDTA</b>	Ethylene di amine tetra acetic acid
<b>ELISA</b>	Enzyme linked Immunosorbent assay
<b>I-ELISA</b>	Indirect Enzyme linked Immunosorbent assay
<b><i>Is711</i></b>	Brucella species gene
<b>MRT</b>	Milk ring test
<b>NCBI</b>	National Center for Biotechnology Information
<b>OIE</b>	Office international des epizooties
<b>PCR</b>	Polymerase chain reaction
<b>RT-PCR</b>	Real time polymerase chain reaction
<b>RBPT</b>	Rose Bengal plate test
<b>RBT</b>	Rose Bengal test
<b>SAT</b>	Serum Agglutination Test
<b>TIVSS</b>	Type 4 secretion system

<b>TBE</b>	Tris Borate EDTA
<b>TSA</b>	Trypticase soya agar
<i>ure</i>	Urease enzyme gene
<i>virB</i>	Transcriptional regulator gene
<i>vjbR</i>	Quorum sensing related Transcriptional regulator gene
<b>VSVRI</b>	Veterinary serum and vaccine research institute
w/v	Weight per volume
<i>ZnuA</i>	Zinc uptake gene

## 5-Summary

A total of 200 samples of raw milk (100 from El-Santa city, Gharbia governorate, 100 from Sidi\_Salem city, Kafer El-Sheikh governorate) were collected from milk shops throughout one year from February 2016 to February 2017.

Sample divided into 3 subsamples. The first 10 ml for milk ring test (MRT), the second 20 ml for bacteriological examination while the third one 10 ml for real time polymerase chain reaction (PCR). Milk samples used for MRT and bacteriological examination were stored at 4°C while milk samples used for PCR assay stored at -20°C till be examined.

The obtained results could be summarized as the following:

### 1. Milk Ring Test (MRT)

MRT was conducted at *brucella* lab, AHRI, Dokki on 200 milk samples. Incidence of *brucella* in milk samples by MRT was 7.0% and 8.0% in El-Santa and Sidi\_Salem respectively in summer and winter seasons with a total positive percent 15% and with 56% sensitivity and 96% specificity.

### 2. Bacteriological examination

#### 2.1. Direct examination of colonies

By culturing on *brucella* specific media suspected *brucella* colonies appeared on 5 culture agar plates showed pale honey color. Incidence of *brucella* in milk samples by culture method was 1.0% in El-Santa city and 1.5% in Sidi\_Salem city with 12% sensitivity and 100% specificity.

### 2.2. Microscopic examination

Smears were made from suspected growths and stained with Gram's stain. The *brucella* organisms appear as Gram negative coccobacilli with Gram's stain.

### 2.3. Biochemical tests for identification of *brucella* species

All isolated bacteria were positive urease test and catalase test while they were negative for H<sub>2</sub>S production test and CO<sub>2</sub> requirements so they were identified as *brucella melitensis*. The results showed high frequency of *brucella melitensis*.

### 3. Detection of *brucella* by Real time PCR from milk samples

RT-PCR assay was conducted to confirm the presence of Genus-specific *L-glutamine 2-deoxy scylloinosose amino transferase gene*, PCR amplified *brucella* specific DNA extracted from 200 milk samples and showed 43 samples were positive. Assays were performed in three runs in duplicate. Cycle threshold (CT) values below 40 cycles were interpreted as positive. Incidence of *brucella* in milk samples by RT-PCR was 10.5% and 11% in El-Santa and Sidi\_Salem respectively in summer and winter seasons with a total percent 21.5%.

### 4. Detection of virulence genes (*Ure*, *bvfA* and *virB* genes) in DNA extracted from milk and *brucella* isolates by conventional PCR

Virulence genes were detected in DNA extracted from 43 milk samples and 5 culture plates as well. The *ure*, *bvfA* and *virB* genes were amplified at 2100bp, 1282bp and 881 bp respectively, Of 43 positive milk samples, 38 (88.0%) samples had *Ure* genes, 34 (79.0%) samples had *bvfA* genes and 32 (74.0%) samples had *virB* genes. Detection of virulence genes

(*Ure* gene, *bvfA* and *virB* genes) in DNA extracted from isolates confirmed that *brucella* species isolated from milk samples were mainly *brucella melitensis*.