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LIST OF ABBREVIATIONS

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

TBE	Tris Borate EDTA
TSA	Trypticase soya agar
ure	Urease enzyme gene
virB	Transcriptional regulator gene
vjbR	Quorum sensing related Transcriptional regulator gene
VSVRI	Veterinary serum and vaccine research institute
w/v	Weight per volume
ZnuA	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_2S production test and CO_2 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 scylloinososе 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*.