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Immunological and Molecular Detection of Human Brucellosis Patients in Muthanna province

A thesis
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By

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Abstract

One hundred positive rose Bengal test samples and 50 samples from healthy individuals as the control group was gathered from human blood depended on age, gender, region, taking medication, and smoking, during the extended period from December/ 2015 to July/ 2016 in Al Hussein Teaching Hospital of Muthanna Province. *Brucella melitensis Rev1* was isolated by Lysis-centrifugation method, and diagnosis of that strain was done by the Api-20E system, Vitek 2 System, conventional Polymerase Chain Reaction (PCR), Serotyping, and Biotyping. Antibiotic sensitivity test done on growing colonies of that strain by disk diffusion method. The best treatments for *Brucella melitensis Rev1* are Gentamicin and Tobramycin. Real Time Polymerase chain reaction technique is done on DNA samples extracted from 100 positive rose Bengal fresh blood samples, that is done by using bcsp31 and omp2 as diagnostic genes, and SYBR Green I dye. The results showed 38 (38%) samples were positive and 62 (62%) were negative for both genes omp2 and bcsp31. So the Real Time Polymerase chain reaction considers as a golden test in specificity and sensitivity, comparing with other parameters in the current study. 2-mercaptoethanol test done to confirm the rose Bengal test addition, differentiate between chronic and acute infections. Positive 2-Mercaptoethanol test samples were 64 (64%) and negative samples were 36 (36%) from positive Rose Bengal test samples, the number of acute infections were 17 (17%) and chronic infections were 47 (47%), at the highest sensitivity (92%), lowest specificity (53%). Indirect enzyme-linked immunosorbent assay IgG did to diagnosis human brucellosis, The results of an indirect ELISA IgG assay excluded the suspected cases ($ELISA_{ex}$) were 26 (26%) positive cases and 74 (74%) negative cases, at highest specificity (96%), lowest sensitivity (63%). The results of an indirect ELISA IgG included the suspected cases ($ELISA_{in}$) were 41 (41%) positive cases and 59 (59%) negative cases, at the intermediate estimations for sensitivity (73%) and specificity (79%). The nitroblue tetrazolium (NBT) test done to estimate the phagocytic activity in positive rose Bengal test patients, The results of (NBT) test

showed a significant increase ($P<0.05$) in phagocytic activity (266%) in the blood of patients who positive rose Bengal test, compared with (112%) in the blood of healthy individuals as a positive control. The MTT assay used for evaluating the lymphocyte transformation index % of peripheral blood leukocytes in positive rose Bengal test patients. The results of MTT assay showed a significant increase ($P<0.01$) in the lymphocyte transformation index (209%) in the lymphocytes of positive rose Bengal test patients, compared with (59%) in the lymphocytes of healthy individuals as a positive control. The total and differential count of the WBC estimate by the system (Sysmex/Japan). The results showed decreases in WBC, neutrophils, monocyte, and increase in lymphocyte at significant ($P<0.05$) in most patients with positive rose Bengal test.

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List of Abbreviations

Abbreviation	Terms
2-MET	2-Mercaptoethanol Test
ADCC	Antibody-Dependent Cell-intervened Cytotoxicity
APCs	Antigen Presenting Cells
bcsp31	*Brucella* cell surface protein 31
BCV	*Brucella* Containing Vacuoles
CBC	Complete Blood Cell count
CD4+ T cell	Cluster of differentiation 4 (T-helper cell)
CFT	Complement Fixation Test
Cgs	Cyclical β-1,2-Glucan synthase
CITA	Centro de Investigación y Tecnología Agroalimentaria
CTLs	Cytotoxic T lymphocytes
CβG	Cyclic β-1,2 glucan
PrPc	cellular prion protein
DCs	Dendritic Cells
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
$ELISA_{ex}$	ELISA excluded the suspected cases
$ELISA_{in}$	ELISA included the suspected cases
ER	Endoplasmic Reticulum
ERES	Endoplasmic Reticulum Exit Site
GTPases	Guanosine 5'-Triphosphatases
HSP60	Heat Shok protein 60
IELISA	Indirect Enzyme Linked Immunosorbent Assay
IFNγ	Interferon gamma
IL-10	Interleukin-10
LAVs	Live Attenuated Vaccines
LAMP-1	lysosomal associated membrane protein 1
LPS	Lipopolysaccharide
LSD	Least significant differences

MAT	**Microagglutination Test**
MHC-II	**Major Histocompatibility Complex Class II**
MR-VP	**Methyl Red – Voges-Proskauer**
NAD	**Nicotinamide-Adenine Dinucleotide**
NBT	**Nitroblue Tetrazolium**
NK	**Natural killer**
NKT	**Natural killer T cells**
NPV	**Negative Predictive Value**
OD	**Optical Density**
omp2	**Outer Membrane Proteins 2**
PAMPs	**Pathogen-Associated Molecular Patterns**
PBMCs	**Peripheral blood Mononuclear cells**
PBS	**Phosphate Buffered Saline**
PPV	**Positive Predictive Value**
qPCR	**Quantitative Real Time- Polymerase Chain Reaction**
RBPT	**Rose Bengal plate test**
RBT	**Rose Bengal test**
ROS	**Receptive Oxygen Species**
RPMI -1640 medium	**Roswell Park Memorial Institute**
RT-PCR	**Real Time- Polymerase Chain Reaction**
SAT	**Serum agglutination Test**
SDA	**Serum–Dextrose Agar**
SRs	**Scavenger Receptors**
STAT	**Standard Tube Agglutination test**
T4SS	**Type IV Secretion System**
TcpB	**Area Containing Protein B**
TGF	**Transforming Growth Factor**
TIR	**Toll-Interleukin-1 Receptor**
TLR2	**Toll-like Receptor 2**
TLR4	**Toll-like Receptor 4**
TNFα	**Tumor Necrosis Factor- Alpha**
TSA	**Tryptose (or Trypticase) –Soy Agar**
WBC	**White Blood Cell**

WHO	**World Health Organization**
αβTCR	**Alpha beta T Cell Receptor**
γδ T cells	**Gamma delta T cells**