

Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

Name: Samah Fikry Mohamed Ali

Nationality: Egyptian

Date and place of birth: 23/1/1972 Giza

Degree: Ph.D.

Specialization: Bacteriology-Immunology-Mycology

Supervisors:

Prof. Dr. Wagih Armanious Gad El-Said

Professor of Microbiology

Faculty of Veterinary Medicine-Cairo University

Dr. Mahmoud El-Said Hashad

Assistant Professor of Microbiology

Faculty of Veterinary Medicine-Cairo University

Dr. Zeidan Mohamed Kholeaf

Head Researcher- Animal Reproduction Research Institute

Title of thesis: "Detection assays for *Brucella species* associated with reproductive problems in cattle and buffaloes using PCR-based tests"

Abstract

In this study, two multiplex PCR assays were introduced and optimized for brucellosis diagnosis. The first assay was able to differentiate between the vaccine strains (S19 & RB51) and other Brucellae while the other one was able to differentiate between the most common virulent Brucella species (*B. abortus* biotypes 1, 2 & 4 and *B. melitensis*). These assays were successfully applied on Brucella references, vaccinal and field isolate strains as well as clinical samples (milk and aborted foeti).

Contents

NO	Items	Page
1.	Introduction.	1
2.	Review of literature.	5
	2.1. History of brucellosis.	5
	2.2. Brucellosis: a livestock threat and zoonotic disease	8
	2.3. Incidence of brucellosis in Egypt.	13
	2.4. Diagnosis of Brucella infection and its common problems	19
	2.4.1. Problems encountered with isolation and identification of Brucella species.	19
	2.4.2. Problems encountered with serodiagnosis of brucellosis.	20
	2.5. Recent methods for diagnosis of brucellosis.	24
3.	Materials and methods.	33
	3.1. Materials.	33
	3.1.1. Brucella reference strains.	33
	3.1.2. Other bacterial strains.	33
	3.1.3. Clinical samples.	34
	3.1.4. Materials used for immunological assays.	34
	3.1.5. Bacteriological media.	35
	3.1.6. Stains.	36
	3.1.7. Dyes used for typing of Brucella.	36
	3.1.8. Diagnostic Brucella monospecific antisera.	37
	3.1.9. Chemicals and reagents used for bacteriological identification.	37
	3.1.10. Materials used for DNA extraction, PCR and agarose gel electrophoresis.	37
	3.1.11. Equipments.	42
	3.2. Methods.	42
	3.2.1. Sampling.	43
	3.2.2. Immunological tests.	44
	3.2.2.1. Rose Bengal Plate test.	44
	3.2.2.2. Standard tube agglutination test.	44

	3.2.2.3. Milk ring test.	45
	3.2.3. Isolation of Brucella from clinical samples	46
	3.2.4. Identification of primary isolates of Brucella.	46
	3.2.5. DNA extraction.	49
	3.2.6. Visualization of the extracted DNA.	51
	3.2.7. Quantitation of the extracted DNA.	52
	3.2.8. DNA amplification by polymerase chain reaction	52
	3.2.9. Procedures adopted to avoid cross contamination and carry over contamination during PCR.	60
	3.2.10. Visualization of PCR products	60
	3.2.11. Specificity of PCR assays.	61
4.	Results.	62
	4.1. Serological and immunological tests.	62
	4.2. Isolation of Brucella organisms from clinical samples.	63
	4.3. DNA extraction from bacterial cultures and different clinical samples.	66
	4.4. PCR amplification assays for detection and identification of Brucella in culture and clinical samples.	67
	4.4.1. PCR assays on DNA extracted from Brucella references, vaccinal as well as field isolates.	67
	4.4.1.1. PCR assay 1.	67
	4.4.1.2. PCR assay 2	68
	4.4.1.3. PCR assay 3.	69
	4.4.1.4. PCR assay 4.	70
	4.4.1.5. PCR assay 5.	71
	4.4.1.6. PCR assay 6.	72
	4.4.1.7. PCR assay 7.	73
	4.4.2. Application of PCR assays on DNA extracted from different clinical samples.	75
	4.4.2.1. Milk samples.	75
	4.4.2.2. Stomach content of aborted foeti.	76
	4.4.2.3. Whole blood samples.	78

	4.5. Specificity of different primers used in the study.	79
5.	Discussion.	80
6.	Summary.	97
7.	References.	100
	Arabic summary.	

List of Abbreviations

- A₂₆₀**: OD at wave length 260.
A₂₈₀: OD at wave length 260.
AMOS: A (abortus), M (melitensis), O (ovis) and S (suis).
B. : Brucella.
BAPAT: Buffered Acidified Plate Antigen Test.
bp: base pair.
BPDA: Bovine Pathogen Detection Assay.
CFU: Colony Forming Unit.
DNA: Deoxyribonucleic acid.
dNTPs: deoxy nucleotide triphosphates.
ELISA: Enzyme Linked Immunosorbent Assay.
ELS: erythrocyte lysis solution
ERIC: Enterobacterial Repetitive Intergenic consensus.
IgG: immunoglobuline G.
IS711: Insertion sequence 711.
KDa: kilodalton.
LIPA: line probe assay.
M: molar.
MRT: Milk Ring Test.
N: normal.
NET: N (Na Cl), E (EDTA) and T (Tris).
NLB: nucleic lysis buffer
NLB: nucleic lysis buffer.
OIE: Office International Des Epizootic.
Omp2A gene: outer membrane protein 2A gene.
PCR: polymerase chain reaction.
Pst 1: restriction enzyme.
RB51: *B. abortus strain RB51* (vaccine strain).
RBPT: Rose Bengal Plate Test.
REP: Repetitive Extragenic Palindromic sequence.
RFLP: Restriction fragment length polymorphism.
S19: *B. abortus strain 19* (vaccine strain).
SAT: Slow Tube Agglutination Test.
SDS: sodium dodocyle sulphate.
16 S rRNA: 16 subunit ribosomal ribonucleic acid.