List of contents

1.Introduction and aim of the work. 1

2.Review of literature. 5

2.1. Brucella and Brucella antigenicity. 5

2.2. Monoclonal antibody approaches. 16

2.3. ELISA using monoclonal antibodies for genus Brucella. 28

3. Material and methods. 49

3.1. Material. 49

3.1.1. Strains. 49

3.1.2. Animals. 49

3.1.3. Cells. 49

3.1.4. Serum samples 49

3.1.5. Chemicals. 50

3.1.6. Plastic wares. 52

3.1.7. Equipments and utensils. 52

3.1.8. Media and working solutions. 53

3.2. Methods 63

3.2.1. Preparation of Brucella cultures. 63

3.2.2. Preparation of LPS antigens. 64

3.2.3. Production of hybridoma in vitro. 66

3.2.3.1. BALB/c mice immunization. 66

3.2.3.2. Myeloma cells preparation. 66

3.2.3.3. Feeder cell layer preparation. 67

3.2.3.4. Preparation of lymphocyte cell suspension. 68

3.2.3.5. Fusion protocol. 69

3.2.3.6. ELISA screening. 72

3.2.3.7. Cloning by limiting dilution. 73

3.2.4. Production of monoclonal antibodies in vivo. 76

3.2.5. Characterization of the prepared monoclonal antibodies 78

3.2.5.1. Western blotting. 78

3.2.5.2. Titration of the monoclonal antibodies in the ascetic fluid. 79

3.2.5.3. Specificity of the monoclonal antibodies. 79

3.2.6. Filed application of the prepared monoclonal antibodies 80

using c-ELISA

4. Results. 82

4.1. Characterization of the prepared antigens. 82

4.2. Concentration of the prepared cultures. 84

4.3. Titration of antibody in the immunized BALB/c mice. 86

4.4. Fusion results. 88

4.5. Characterization of the prepared monoclonal antibodies. 92

4.6. Results of c-ELISA using the prepared monoclonal antibodies. 102

5. Discussion and conclusion. 108

6. Summary. 117

7. References. 120

8. Arabic summary

List of Abbreviations

BALB/c mice	Bagg albinos / colored locus mice
B.abortus	Brucella abortus
B.canis	Brucella canis
B.melitensis	Brucella melitensis
B.neotomae	Brucella neotomae
B.ovis	Brucella ovis
B.suis	Brucella suis
c-ELISA	Competitive Enzyme linked Immunosorbent Assay
CFT	Complement Fixation Test
C RPMI 1640	Complete Rosewell Park Memorial Instiute Medium
D.dist. H_2O	Double distelled water
DMSO	Dimethyl sulfoxide
EDTA	Ethylene Diamene Tetra Acetic acid
ELISA	Enzyme Linked Immunosorbent Assay
FCS	Faetal Calf Serum
HAT	Hypoxanthine Aminopetrin Thymidine
HGPRT	Hypoxanthine Guanine Phospho Ribosyl Transferase
HT	Hypoxanthine Thymidine
i-ELISA	Indirect Enzyme Linked Immunosorbent assay
I-RPMI	Incomplete Rosewell Park Memorial Institute Medium
Kda.	Kilo Dalton

LPS	Lipopolysaccharide
LPS-A	A-epitope in lipopolysaccharide
LPS-M	M-epitope in lipopolysaccharide
MoAb	Monoclonal antibody
MoAb-A	Monoclonal antibody against A epitope of Brucella lipopolysaccharide
MoAb-M	Monoclonal antibody against M epitope of Brucella lipopolysaccharide
OD	Optical Density
OPD	Orthophenyl diamne
OPI	Oxalacetate Pyruvate Insulin
O-PS	O chain polysaccharide
PBS	Phosphate Buffer Saline
PEG	Polyethylene glycol
Pristane	2,6,10,14-tetramethyl pentadecane
RBT	Rose Bengal test
SDS	Sodium Deodocyl Sulphate
SDS-PAGE	Sodium Deodocyl Sulphate PolyAcrylamide Gel electrophoresis
TEMED	N,N,N,N Tetramethyl diamine
µl	Microliter

6.Summary

Brucellosis not only a zoonotic disease , but also causes heavy economic losses in animal industry , that give the high importance of its control which depend mainly on accurate serological diagnosis and vaccination .

Among all serological tests that had been developed , cELISA could overcome the problem of cross reaction that occurred from other bacteria sharing identical epitope , beside being rapid and highly sensitive .

This assay performed by using monoclonal antibody against A and M epitopes from lipopolysaccharides of S19 and Rev1 respectively , as serological responses following infection or vaccination directed predominantly against the lipopolysaccahrides.

The prepared lipopolysaccharides have been characterized by SDS-PAGE , where the LPS-A and LPS-M showed molecular weight of 40 kDa.. and 50 kDa.. respectively . Then the dose used for immunization determined as at dilution 1/75 for S19 and 1/100 for Rev1 .

Six BALB/c mice were immunized by S19 and LPS-A and another six BALB/c mice immunized by Rev1 and LPS-M. Antibodies produced gave successful values at 1/3200 dilution from sera taken randomly from each group of mice .

Splenocytes from the immunized mice have been fused with myeloma cells of high viability and HGPRT deficient . The resultant cells having the properties of immortality and antibody production.

LPS-A and LPS-M fusion plates revealed six and nine positive clones respectively , these clones were diluted by limiting dilution , where three positive clones from each fusion emerged , but only one from each was successfully used , namely BA1B5 for LPS-A fusion and BM1F7 for LPS-M fusion.

The two prepared clones were injected in three mice for each , primed with pristane and the ascetic fluid were harvested and purified.

BA1B5 gave high reactivity only with S19 and LPS-A till dilution 1/1000 , while that of BM1F7 gave only with Rev1 and LPS-M till 1/5000 dilution. None of the prepared antibodies gave significant reaction with *Yersinia enterocolitica 0:9* , *E.coli* or *Salmonella tyhpimurium.*

The prepared monoclonal antibodies were blotted by western blot assay . The monoclonal antibody prepared against BA1B5 gave only one band with S19 and LPS-A , while BM1F7 gave only one band with Rev1 and LPS-M indicating that these prepared antibodies were monoclonal and specific only with their corresponding antigens.

c-ELISA was done using the prepared monoclonal antibodies on 18 sera samples , 6 from infected cattle with B.melitensis biovar 3 , 4 from cattle vaccinated with S19 , 4 from sheep vaccinated by Rev1 vaccine and 4 from sheep free from brucellosis and not vaccinated.

High inhibition percents were obtained using BA1B5 with the infected samples , and also with the samples vaccinated with S19 . while using BM1F7 gave high inhibition percent with the infected samples as well as the Rev1 vaccinated samples . Rose bengal test was done on all serum samples , highest titer were detected in the S19 vaccinated samples , while the infected samples gave higher concentrations than the Rev1 vaccinated samples.

Also i-ELISA was done on the same samples using LPS-A and LPS-M as coating antigens . Both i-ELISA and rose bengal test gave results agreed to those obtained by c-ELISA , but more advantages found in c-ELISA as it could overcome any false positive reaction from Yersinia enterocolitica 0:9 infection and it could done on any samples regardless to the species.