Abstract

Some farms may have mixed animals, (cows, goats, sheep & camel). It has not been well described in the literature as to whether cross infection of different species of *Brucella* occurs, when these animals are kept together, particularly in the case of camels, sheep and goats in which the main virulent organism is the same *(Brucella meliensis*). Most of the previous studies undertaken followed serological testing for the investigation of *Brucella* prevalence and epidemiology to investigate this problem. In the present study serological tests were carried out for screening and bio-epidemiological techniques using PCR for confirmation. The objective is to study the incidence of brucellosis in different types of mixed animal herds containing sheep, goats and camel to evaluate the significance of brucellosis problem and the pattern of the disease transmission and shedding of organisms in milk of carriers and resistant animals. .

The farms were categorized in two different types namely, agro pastoralist, and Feed-lot. A total of 854 blood samples were collected during the year 2005. Sera was separated and tested using monospecific antigen.

The over all prevalence rate in which this study was conducted (Al Quaseem area) according to RBBT was found to be 5.9%.

The incidence rates in different mixed farming systems were as follows:

A. The incidence rate in Feed lot farms was 8.4%

B. The incidence rate in the Agro pastoralist farms was 4.9 %

The incidence in different animals within the two different farming systems was found 4.1%in sheep, 9.7% in goats 5% in cow, 2% in camels

The predominant species of *brucella* using monospecific antigen was found to be
B. melitensis with some few cases of *B. abortus*

The study revealed that there was a high incidence rate in the feed lot system particularly in the resident animals (goats) in this system.

The *brucella* organism was isolated from both stomach content of aborted sheep fetus and detected by PCR.

The study reveals that PCR technique could be used as well as bacteriological testing for confirmation of *brucella* infection

Contents

No.	Items	Page
1	**Introduction.**	
2	**Literature review.**	
	2.1. History of brucellosis.	
	2.2. Geographic distribution.	
	2.3. Host range	
	2.4. Causative agent	
	2.4.1. *Brucellae* Cellular Structure and Composition	
	2.4.2. Growth Characteristics.	
	2.4.3. Taxonomy of species.	
	2.5. Clinical signs of brucellosis in different animal species.	
	2.5.1. **Bovine brucellosis**.	
	2.5.2. Ovine brucellosis.	
	2.5.3. Brucellosis in camel.	
	2.5.4. Brucellosis in pigs.	
	2.5.5. Brucellosis in horses.	
	2. 5.6. Brucellosis in dogs.	
	2.6. Zoonosis of the disease (Human brucellosis)	
	2.7. Diagnosis.	
	2.7.1. Clinical signs.	
	2.7.2. Bacteriological examination.	
	2.7.3. Serological tests.	
	2.7.3.1. Milk ring test.	
	2.7.3.2. Whey agglutination test (WAT)	
	2.7.3.3. Serum agglutination test (SAT).	
	2.7.3.4. Complement fixation test (CFT).	

	2.7.3.5. Rose Bengal plate test (RBPT)	
	2.7.3.6. Coombs antiglobulin test: (CAGT).	
	2.7.3.7. Enzyme linked immunosorbent assay (ELISA).	
	2.7.3.8. Skin delayed type hypersensitivity (SDTH).	
	2.7.3.9. Supplementary tests.	
	2.7.3.9.1. The disulphide reduction.	
	2.7.3.9.2. The heat inactivation test.	
	2.7.3.9.3. The Rivanol test.	
	2.7.4. Polymerase chain Reaction (P.C.R)	
	2.8. Resistance to infection.	
	2.9. Survival of *brucella* in the environment.	
	2.10 Transmission and dissemination of *brucella* organism.	
	2.11 Immune response.	
	2.12. Control of brucellosis.	
	2.12 .1. Control of bovine brucellosis.	
	2.12.2. Control of ovine brucellosis.	
	2.12.3. Control of brucellosis in other animals	
	2.13. Treatment of human infected with brucellosis.	
	2.8. Resistance to infection.	
3	**MATERIALS AND METHODS**	
	3.1 Source of samples.	
	3.1.1. Agro pastoralist system.	
	3.1.2 Feed lot system.	
	3.2. Materials.	
	3.2.1. Materials used for immunological assays.	
	3.2.2. Bacteriological media.	
	3.2.3. Stains.	

	3.2.4. Dyes used for typing of *Brucella*.	
	3.2.5. Monospesific antisera	
	3.2.6. Chemicals used for bacteriological Identification.	
	3.2.7.Material used for DNA extraction, PCR assay and agarose gel electrophoresis.	
	3.3. Equipments	
	3.4. Methods.	
	3.4.1. Sampling.	
	3.4.2. Serological tests.	
	3.4.2.1. Rose Bengal test.	
	3.4.2.2. Milk Ring Test (MRT).	
	3.4.3. Bacteriological Methods.	
	3.4.3.1. Isolation of *Brucella* organism.	
	3.4.3.2. Identification of *Brucella* organism.	
	3.4.4. Molecular methods.	
	3.4.4.1. DNA extraction.	
	3.4.4.2. Visualization of extracted DNA.	
	3.4.4.3. Quantitation of extracted DNA.	
	3.4.4.4.Polymerase Chain Reaction (P.C.R) DNA amplification by Polymerase chain reaction	
	3.4.4.5. Procedures adopted to avoid cross contamination and carryover contamination during PCR.	
	3.4.4.6. Visualization of PCR products.	
	3.2.5. Monospesific antisera.	
	3.2.6. Chemicals used for bacteriological Identification.	
	3.2.7. Material used for DNA extraction, PCR assay and agarose gel electrophoresis.	
4	**Results**	
5	**Discussion**	
6	**References**	

List of Tables

No.	Table title	Page
1	**Table 1: Growth requirements, biochemical characteristics of different species and biotypes of *brucellae*, and host range.**	
2	**Differential characteristics of *Brucella* Phages.**	
3	**Table 3: Biovar differentiation of *Brucella* species involved in ovine caprine as well as bovine *brucellosis.***	
4	**Table4: Differential characteristics of *brucella* species involved in cattle sheep and goat *brucellosis***	
5	**Table 5: Oligonucleotide primers used for *Brucella* DNA amplification, their references, expected product sizes and species specificity.**	
6	**Table 6: No. of samples taken for culturing, their type and the species of animal from which they were taken.**	
7	**Table 7: PCR assay1, reaction components and amplification condition.**	
8	**Table 8: PCR assay 2 reaction components and amplification conditions.**	
9	**Table 9: The prevalence rate in different species of animals.**	
10	**Table 10: The prevalence rate in different farming systems**	
11	**Table 11: The prevalence rate in lactating, dry, and young animals.**	
12	**Tabble12: The *Brucella* types of infection according to the mono specific antigen .**	
13	**Table13: Cultural, biochemical and serological identification of *Brucella* isolates recovered in the study.**	
14	**Table 14: Reaction of different *Brucella* reference, vaccinal and isolate strains with various primer sets used in the study**	

List of figures

No	Figure title	Page
1	**Fig. 1.1: DNA double helix.**	
2	**Fig. 1.2: The structure and ring numbering of the Bases.**	
3	**Fig.1. 3: Three Nucleotide Pieces of single stranded DNA.**	
4	**Fig. 1.4.: DNA band visualized in agarose gel**	
5	**Fig.1.5: The PCR components**	
6	**1.6 PCR product of 223bp using PCR assay.**	

List of Abbreviations

A_{260}: OD at wave length 260.

A_{280}: OD at wave length 260.

B.: Brucella**.**

BAPAT: Buffered Acidified Plate Antigen Test**.**

Bp: Base pair.

CFU: Colony Forming Unit**.**

DNA: Deoxyribonucleic acid.

DNTPs: Deoxy nucleotide triphosphates**.**

ELISA: Enzyme Linked Immunosorbent Assay**.**

FB51: B. Abortus strain RB51

IgG: Immunoglobulin G.

IS711: Insertion sequence 711.

KDa: Kilo Dalton**.**

LIPA: Line Probe Assay.

M: Molar.

MRT: Milk Ring Test**.**

NET: N (Na Cl), E (EDTA) and T (Tris).

OIE: Office International Des Epizootic**.**

Omp2A gene: Outer Membrane Protein 2A gene**.**

PCR: Polymerase Chain Reaction**.**

RBPT: Rose Bengal Plate Test.

REP: Repetitive Extragenic Palindromic sequence**.**

RFLP: Restriction Fragment Length Polymorphism

S19: B. abortus strain 19.

SAT: Serum Agglutination Test**.**

PCR: polymerase chain reaction

bp : base pair

MW: molecular weight marker