Molecular Characterization and Epidemiological Investigation of Brucellosis in Sharqyia in Egypt

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Introduction

Brucellosis is a wide spread disease among animals and human and of a major economic importance due to abortions, decrease milk yield, temporarily or occasionally permanent sterility[1]. In Egypt, *Br. abortus* was the commonly isolated species until the beginning of 1970s [2]. In the last years, *Br. Melitensis* become the most common strain prevalent in animals in Egypt [3,4]. The organism can be detected by polymerase chain reaction (PCR) in blood, semen and abomasal fluid of aborted fetuses and in compare to culture method, PCR has more sensitivity and specificity [5]. The aim of the present study was to evaluate of the vaccination program and immune response of vaccinated animals in Sharqyia province.

Materials and methods

Study animals: A total of 4772 lactating and non-lactating cows from were examined for *brucella*.The present study was conducted during the period from 2008 to 2011 in Sharqyia Province.

Samples and sample processing: Blood and milk were collected from private farms and individual distributed animals on different districts in Sharqyia Province to survey the prevalence of brucellosis. Supra-mammary, internal iliac and superficial cervical lymph nodes of adults slaughtered infected cows.

Immunological study: The conventional serological tests including Rose Bengal test (RBT), Wright's sero-agglutination test (SAT) and 2-mercapto-ethanol test (2ME) were performed on sera samples according to standard techniques [6].

Clinical examination and epidemiological studies: A number of 710 cows (lactating, non-lactating and heifers) distributed in two farms in Sharkia province with a history of brucellosis were examined serologically by using of milk ring test (MRT), buffered acidified plate antigen test (BAPAT), rosebengal plate antigen test (RBPT), tube agglutination test (TAT), enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR).

Bacteriological Analysis

Isolation and identification: Blood samples were collected for isolation and identification of bacteria according to Alton et al [6] and serological tests were applied according to Hess [7] and [8]

Bacteriological culture was carried out on specimen from retropharengeal, supramamary, lymph nodes obtained from animal's positive sero-test method described by Alton et al [6] . Biochemical tests, dye sensitivity, exposure to monospecific antisera, suscesptability to antibiotics and lysis by phages were performed on colonies with charateristics typical of genus *brucella*.

Molecular characterization of brucella species isolated from dairy cows: The study was conducted on heifers, pregnant and delivered cows. Blood samples were taken according to the breeding records for comparison among them. They were kept under restricted program for controlling the internal and external parasites. They were also kept under standard level of nutrition, mineral mixture and water were available add libitum.

Bacterial strains: The bacterial strains used in this study were isolated by conventional methods from milk samples, aborted fetuses, and placenta (J-M. verger, Institute National de la Recherche Agronomique, Nouzilly, France. Strain biotyping was performed by standard methods.

Molecular analysis:
Extraction of DNA: Extraction of DNA was carried out according to Donis et al [9].

DNA digestion: Restriction enzymes were used according to the manufactured instructions (Boehringer GmbH, Mannheim, Germany).

Conclusion and future prospects

Our results revealed that the prevalence of *brucella* were 3.06 % of private farms compared to 2.82 % for individual animals. Bacteriological examination revealed that *B. Melitensis biovar-3* was isolated. These reflect the role of cattle in transmission and spreading of *brucella*.

We assumed that the sensitivity of the test would be doubled by selecting duplicated DNA sequences of two gene, we assumed that because of the existing Pst I site polymorphism between *B. melitensis* and *B. abortus*, the test is specific for distinguishing between 2 species .

Finally, it should be focused on the problem of the disease in small ruminants as they played a role in transmission of the disease to eliminate it and reduce the prevalence of the disease among cattle.

References

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Results

Table. 1: The incidence of brucellosis in Lactating, non-lactating and heifers cows in different districts in Sharqyia province.

Source of animals	*No. of animals*	*BAPAT*		*RBPT*		*TAT*		*CFT*		ELISA	
		R	R %	R	R %	R	R %	R	R %	R	R %
Private farms	2802	124	4.42	124	4.42	124	4.42	124	4.42	124	4.4
Individual animals	1970	176	8.9	176	8.9	176	8.9	176	8.9	176	8.9
Total	**4772**	**300**	**6.2**	**300**	**6.2**	**300**	**6.2**	**300**	**6.2**	**300**	**6.2**

Table. 2: Bacteriological isolation for different samples randomly collected from serologically positive animals with brucellosis in Sharqyia province.

Animals	*Total No. of Samples*	*Milk Samples*		Lymph nodes tissue specimen	
		(-) ve	(+) ve	(-) ve	(+) ve
Private farms	25	23	2	19	6
Individual animals	25	21	4	20	5
Total	**50**	**44**	**6**	**39**	**11**

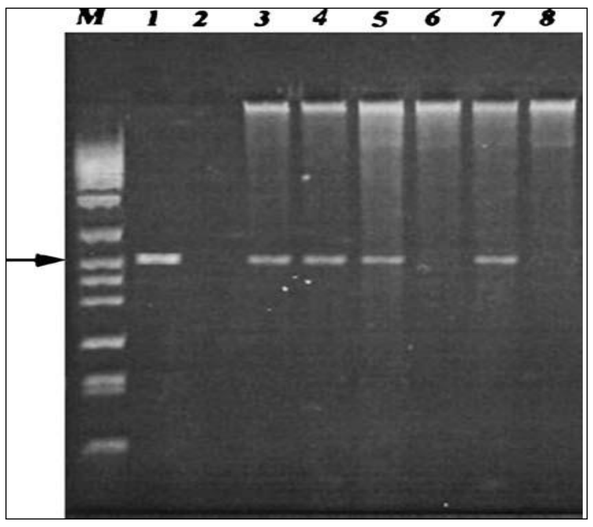
Table. 3: Percentages of abortion rates in private farms distributed in different districts in Sharqyia province.

Year of examination	*No. of ♀*	*Number of aborted ♀*	% of abortion
2008	1030	16	1.55
2009	842	22	2.61
2010	538	17	3.16
2011	392	12	3.06
Total	**2802**	**67**	**2.39**

Table. 4: Percentages of cows suffering from brucellosis associated with some breeding troubles from both private farms and individual animals.

Breeding abnormalities	*No of investigated cows*	*Private Farms*		Individual animals	
		No of infected cows	%	No of infected cows	%
Retained placenta	150	5	3.3	4	2.7
Difficult birth	150	7	4.7	6	4
Ret. & Diff. birth	150	3	2	2	1.3
Endometeritis	150	4	2.7	3	2
Repeat breeder	150	0	0	1	0.7

Figure. 1: Percentages of cows suffering from brucellosis associated with some breeding troubles from both private farms and individual animals.



Legend: Lane M standard DNA marker, lane 1 positive control, lane 2 negative control, Lanes 3, 4 and 5 positive blood sample DNA PCR; lane 6 and 8 negative Blood sample, lane 7 positive vaccine DNA PCR. The 720-bp PCR product is indicated.

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