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Seroprevalence and Molecular Epidemiology of Brucellosis in Cattle in Egypt

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Abstract

The study was applied on 4772 lactating and non-lactating cows distributed on different districts in Al Sharqia Governorate. Sera were collected from animals during routine diagnosis and control program. The results of screening tests Buffer acidified plate antigen test (BAPAT), Rose Bengal plate test (RBPT), Tube agglutination test (TAT), Complement fixation test (CFT) and indirect enzyme linked immunosorbent assay gave 124 and 176 seroreactive animals by incidence of 4.42% and 8.91% in private farms and individual cases respectively. 37 (29.8%) and 97 (55.1%) isolates of *Brucella melitensis biovar* 3 were recovered from 124 and 176 seroreactive animals respectively. In seroreactive cows, *Brucella melitensis* biovar 3 was isolated from 36% and PCR yielded expected products in 40%. In conclusion, more attention should be paid to the role of *Brucella melitensis* biovar 3 in brucellosis in cattle during the application of national program of *brucella* control and eradication.

Keywords: Prevalence; *Brucella melitensis*; Seroreactive; Cattle; Egypt

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, B. abortus was the commonly isolated species until the beginning of 1970s [2]. In the last years, B. melitensis become the most common strain prevalent in animals in Egypt [1,3,4]. Brucella is a facultative, intracellular, gram negative, bacterial pathogen and the etiologic agent of brucellosis, important zoonosis with a nearly worldwide distribution [5]. distribution of the disease appears to be correlated with high animal densities associated with winter feeding [6]. Clinical symptoms of brucellosis are and its diagnosis in sheep is currently based on serological and microbiological tests [7,8]. A variety of antimicrobial drugs have activity against *Brucella*, however, the results of *in vitro* susceptibility tests do not always correlate with clinical Bacteriological isolation of B. melitensis and/or positive blood culture soon the infection are common laboratory procedures that are used for diagnosis. However, these procedures are not always successful as they are complicated and represent a great risk of infection for laboratory technicians [9,10]. Serological tests can also be used for diagnosis of Brucella spp. infection via detection of antibodies in serum [11].

In addition, the organism can be detected by polymerase chain reaction (PCR) in blood, semen and abomasal of aborted fetuses and, compare to culture method, PCR has more sensitivity and [8,12]. Recently, PCR assay has been used for detection of Brucella spp. It is a promising alternative for conventional bacteriological techniques due to its speed, safety, high sensitivity and In Egypt, control of Brucellosis is yet a task since it had been diagnosed by Ahmed [13], despite the exhaustive and concepts of approach; this is mainly due to the very high cost and the wide range of maintenance factors of Brucella organisms. aim of the present study was to determine the immune response of vaccinated animals and the presence of Brucella in blood vaccination using serological tests and PCR method.

Materials and Methods

Description of the study area

study was carried out in Al Sharqia Governorate, East of Cairo. region was purposively selected for the study because it's have large numbers of animals and human populations. study was carried out between September 2008 to July 2011. region has warm and cool dry season with considerable variation of rainfall from year to year. area has a long rainy season which extend from November to April and a dry season which extends from May to October.

Animals

A total of 4772 cattle were examined for Brucella. animals were kept under restricted program for controlling the internal and external parasites, vaccination programs and standard level of nutrition; whereas mineral mixture and water were available add libitum.

Study design and samples size estimation

A cross-sectional study was conducted to determine the epidemiology of Brucella infection in animals in districts making up the Al Sharqia region. sample sizes for animals for serological studies, milk samples from cattle for molecular studies, were calculated by the formula of multistage random sampling described by [36]. A random sample of villages of Al Sharqia Governorate was done, using a table of random numbers, from a sampling frame comprised of a list of all villages in the study area. We collected the samples from private farms and areas for individual animals; we select the number of animals every year.

Samples collection

Cattle were manually restrained and had blood samples taken from the jugular vein using 10 ML plain vacutainer tubes. blood samples from cattle were allowed to clot in a slant position and serum samples were harvested 24 h. harvested serum was transferred to 1.5 ML cryovials and stored in Liquid Nitrogen (LN) before being transferred to the laboratory at the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt where they were stored in an ultradeep freezer (-80 °C) until tested. Cows from each selected households were randomly selected and sampled to obtain a total of 4772 animals. Milk samples were collected under hygienic condition from udders of cattle by hand stripping just prior to milking using sterile screw caped 50 ML falcon tubes. Each sample was composed of representative amount of milk taken from each quarter. Volumes of about 12 ML of milk sample were taken from each quarter to have a total of 50 ML of milk from cattle. First streaps of milk from each quarter were discarded. Blood was collected from lactating cattle in which milk was sampled for *Brucella* DNA detection using 10 ML plain vacutainer tubes for serological study. samples were immediately stored in LN before transferred to the laboratory at the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt to be stored in ultra-deep freezer (-80 °C) until tested.

Epidemiological investigation

Cows of ages and gestation stages, Lactating and non-lactating were examined for abortion and breeding troubles including retained placenta, retained placenta with birth, endometritis and repeat breeder. Data regarding beginning of these troubles were also recorded.

Bacteriological isolation

Blood samples were collected for isolation and of bacteria according to Alton et al. [15] and serological tests were applied according to Hess [17] and Lambert and Amerault [18]. Bacteriological culture was carried out on specimens from retropharyngeal, supramammary lymph nodes obtained from seroreactive animals was described by Alton et al. [15]. Biochemical tests, dye sensitivity, exposure to antisera, susceptibility to antibiotics and lysis by phages were performed on colonies with characteristics typical of genus Brucella.

Serological tests

collected serum samples were examined by BAPAT and RBPT as screening tests. all positive serum samples were further retested by TAT, CFT and ELISA as quantitative tests. antigens of BAPAT, RBPT, and TAT were supplied by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt and performed according to Alton et al. [15]. ELISA antigen was supplied from Synbiotics Europe 2, rue A–Fleming 69007 Lyon-France. Serum samples were performed by ELISA as mentioned by Cardoso et al. [21]. Rose Bengal Plate test (RBPT) was done according to Alton et al. [15] and Morgan et al. [19]. While, plate antigen test (BAPAT) was applied according to [20].In addition, tube agglutination test (TAT) was done according to the method adopted by the central veterinary laboratory (C.V.L.), Weybridge, England as described by Alton [7]. Complement Fixation test (CFT) was done according to Alton et al. [15].

PCR assay

Extraction of DNA was carried out according to [22]. PCR and oligonucleotide primers: the brucella Omp 2 gene was used as target DNA. forward primer (p1 {5' TGGAGGTCAGAAATGAAC 3'}) and reverse primer (p2 {3' GAGTGCGAAACGAGCGC 5'}) of an Omp 2 gene segment were obtained from National Bioscience, Inc., Plymouth, Minn. PCR was performed by the method of [23]. A typical reaction mixture contained 50 mM Kcl, 1.5 mM MgCl2, 0.1 % (wt/vol). Triton X-100, 0.2 mg of bovine serum albumin (fraction IV; Sigma) per ml, and mM each of the four deoxyribonucleotides, 100 ng of sample DNA and each oligonucleotide primer. For slide PCR, sample DNA was replaced with brucella that was laid on a glass slide, air dried, and by being heated. A sample of the dried cells was then collected with a needle, the needle was dipped in 10 ul of double-distilled water, and 2 ul from this solution was put in the PCR mixture. Otherwise, sample DNA (2 ul from a bacterial cell suspension in double distilled water boiled at 100 C for 20 min.) was used. Reactions were initiated by adding 0.5 U of Taq polymerase (Appligene, IIIkirch, France).

reaction mixture was covered with 15 ul of mineral oil (Sigma) to prevent evaporation. Following hot start treatment at 95 C for 3 min., PCR was performed with an Eppendorf (Eppendorf, Humburg, Germany) as follow 35 cycles of PCR, with 1 cycle consisting of 20 s at 95 C for DNA denaturation, 1 min at 50 C for DNA annealing, and 1 minutes at 72 C for polymerase mediated primers extension. last cycle included incubation of the sample at 72 C for 7 min. ten micro liters of the product was analyzed with electrophoresis in 1.5 % agarose gel in TEA (20 mM Tris-acetate, 1 mM EDTA {pH 8.0}.

Data Analysis

Excel® 2007 Corporation, One Way, Redmond, 98052-7329, USA) was used in storing data and drawing graphs. Data was analysed using Epi-Info version 7 (CDC Atlanta, USA) and MedCalc® version 13.0.2 (MedCalc Acacialaan 22, B-8400, Ostend, Belgium). Chi square test was performed to calculate P value for the incidence rate of Brucella versus the age groups, sex and species. P value < 0.05 was considered statistically

Results

Serological results

In private farms, 124 out of 2802 (4.42 %) cattle sera tested was positive to RBPT and BAPA. RBPT positive samples were further by the TAT, CFT and ELISA. While in individual animals, 176 (8.93 %) out of 1970 cattle sera tested positive with RBPT. RBPT positive sera were retested with ELISA with 176 (8.93 %) were found positive. was a statistically in seroprevalence between private farms and individuals cattle (P < 0.05). (Table 1).

Animals	No. of animals	BAPAT		RBPT		TAT		CFT		ELISA	
		No	% pos.	No	% pos.	No	% pos.	No	% pos.	No	% pos.

Private farms	2802	124	4.42	124	4.42	124	4.42	124	4.42	124	4.42
Individual animals	1970	176	8.93	176	8.93	176	8.93	176	8.93	176	8.93

Table 1: Prevalence of Brucella antibodies in cattle in Al Sharqia Governorate .

Bacterial isolation

17 out of 50 positive reactors Brucella melitensis biovar 3 was isolated. (Table 2).

Animals	Total no. of samples	No. & % of Brucella isolates	
		Positive	%
Private farms	124	37	29.84
Individual animals	176	97	55.11

Table 2: Bacteriological isolation of seroreactive animals.

PCR results

Ten (0.40) out of 25 milk samples from seropositive cattle tested positive with PCR. While Seven (0.28)out of 25 serum samples from seropositive cattle tested PCR positive. Brucella species detected from cattle was Brucella melitensis biovar 3. All PCR positive results were shown by migration of PCR product to approximately 720-bp for Brucella melitensis fragments (Figure 1 and Table 3).

Test	Positive	Bacterial isolation	PCR
BAPAT	25	9 (0.36)	10 (0.40)
RBPT	25	7 (0.28)	8 (0.32)
TAT	25	7 (0.28)	7 (0.28)
CFT	25	7 (0.28)	7 (0.28)
ELISA	25	7 (0.28)	7 (0.28)

Table 3: Evaluation of diagnostic tests in *Brucella* seroreactive animals.

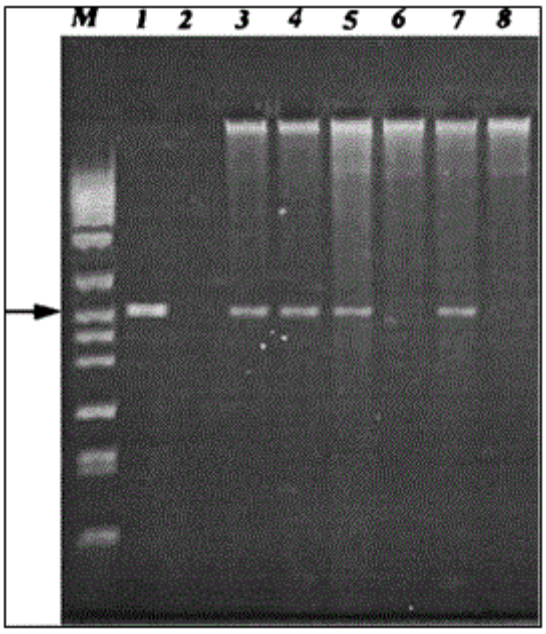

Figure 1: Electrophoretic pattern of PCR product 720-bp bp in 1.5% agarose gel stained with Ethidium bromide. Lane M standard DNA marker, lane 1 positive control, lane 2 negative control, Lanes 3, 4 and 5 positive blood sample DNA PCR; lane 6 and 8 negative Blood sample, lane 7 positive vaccine DNA PCR.

Abortion & Breeding troubles

percentages of abortion in pregnant cows from brucellosis in some private farms were 1.55 %, 2.61 %, 3.16% % and 3.06 % for years 2008, 2009, 2010 and 2011 respectively (Table 4). While, the percentages of abortion in pregnant cows from brucellosis collected from individual animals were 2.29 %, 1.41 %, 2.59 % and 2.83 % for yeas 2008, 2009, 2010 and 2011 respectively (Table 4, 5 and 6).

Year	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

Table 4: Percentage of abortion in private farms.

Year	No. of ♀	No. of aborted ♀	% of abortion
2008	654	15	2.29
2009	496	7	1.41
2010	502	13	2.59

2011	318	9	2.83

Table 5: Percentage of abortion in individual investigated animals.

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

Table 6: Percentage of cows from brucellosis associated with breeding troubles.

Discussion

Brucellosis is a well-documented zoonosis worldwide posing serious public health problems and extensive economic losses [22]. In these areas brucellosis represents a public health issue and its incidence might reach more than 200 cases per 100000 populations [24]. misdiagnosis and under reporting of brucellosis were make the true incidence of brucellosis remains unknown and might extend to 25 times higher than the one [25]. ultimate goals of vaccination are to control disease and reduce or eliminate transmission from reservoir species. To accomplish these goals in ruminants using brucella vaccines, the development of more vaccination mechanisms are need to enhance vaccine

Our results revealed that the antigen reach the immune system and it delivered to the antigen presenting cells are fundamental in the induction of an optimal immune system response. incidence of brucellosis in cows either lactating or none lactating during stages of gestation, and heifer's ones in some private farms and individual animals, the percentages of serologically reactors were 4.42 % and 8.9 % for cows in private farms and individual respectively. results recorded by [26,16] who observed that vaccine used serves to modify the uptake and processing of antigen. Furthermore, [27] suggested that prolonged persistence of the vaccinal strain in the host needed for the development of suitable anti-brucella immunity.

Bacteriological isolation of brucella from milk samples was 8 % and 19 % and 25 % and 31.6 % from lymph nodes of seroreactive animals from private farms and individual cases respectively; this result agrees with [15] who isolate brucella species from milk samples and lymph nodes.

PCR has increasingly been used as a supplementary method in Brucella diagnosis [32]. Recently a molecular biotyping approach has been proposed on the basis of restriction endonuclease polymorphism in the genes encoding the major outer proteins of Brucella membrane [33]. Omp2 gene exists as a locus of two nearly homologous repeated copies that slightly among Brucella species and biotypes [34]. We used previous information to design primers that amplify a 720 bp fragment lanes 3, 4 and 5 shows the positive samples taken from farm vaccination with RB51 vaccine, whereas lane 7 only positive samples collected from second farms, Lanes 6 and 8 were negative for PCR against brucella species.

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 for distinguishing between 2 species [35].

percentages of abortion in pregnant cows from brucellosis in some private farms were 1.55 %, 2.61 %, 3.16% and 3.06 % for years 2008, 2009, 2010 and 2011 respectively. While, the percentages of abortion in pregnant cows from brucellosis collected from individual animals were 2.29 %, 1.41 %, 2.59 % and 2.83 % for yeas 2008, 2009, 2010 and 2011 respectively. results were lower than those reported by [28,29,30] whose recorded 16.1 %, 37.4 %, and 26 %, respectively.

breeding troubles of investigated animals were retained placenta, birth, retained placenta and birth, endometritis and repeat breeder in random investigated animals were 2.7%, 4%, 1.3%, 2% and 0.7 % respectively., results agrees with [15,31,21] who proved that breeding troubles and poor feeding increasing the infection with brucella.

In conclusion, raising goats with large dairy animals is a faulty traditional practice, whereas it may be a source of B. melitensis infection for animals in Egyptian villages. 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.

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