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ENDEMIC SITUATION OF BRUCELLOSIS IN DAIRY FARMS AFTER RB51 VACCINATION

BY

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ABSTRACT

The aim of this study is detection of annually endemic situation of bovine brucellosis in dairy farms after RB51 vaccination allover 4 years (2008- 2011). The abortion rates were 1.16%, 1.19%, 0.92%, 1.27% in 2008, 2009, 2010 and 2011 respectively. While the total prevalence rate were 2.81%, 2.39%, and 2.49% by BAPAT, CFT and ELISA respectively. There are non-significant differences between years by different serological tests. The study concluded that the efficacy of RB51 vaccination program in decreasing the abortion rate and infection, but it failed in elimination of brucellosis which need reevaluation of brucellosis control program components and need further study in brucellosis endemic situation especially in other natural hosts which facilitate maintenance of infection in endemic areas.

INTRODUCTION

Brucellosis is one of the most bacterial zoonotic diseases in the world and cause high economic losses in most animal species among most countries that suffered from brucellosis **Shaw (1906)**. World Health Organization for Animal Health, Paris (OIE) concerning the disease is one of the most dangerous bacterial diseases **(World health Organization, 1998).** Also Brucellosis is highly infectious diseases characterized by enlargement of supramammary lymph nodes and the udder of cows dose not show any growth lesions **(Minas *et al.,* 2005, and Garin-Bastuji *et al.,* 2006)** and storm of abortion induced by brucellosis among cattle **(MacMillan, 1990).**

Brucellosis in animals has been recorded in Egypt since 1939 by **Ahmed (1939)**. Isolation of *Brucella species* from cattle were made by various workers as early as **Zaki, (1948).**

The infestation of animal production during last century with the importation of Friesian cows for establishment of governmental farms with large number of animals lead to an increase of the incidence of Brucellosis in cattle reach to 37% in some of these farms **Refai *et al.*, (1990).**

Brucella abortus strain RB51 (SRB51) is a live, stable, rough mutant of B. abortus strain 2308 that lacks much of the lipopolysaccharide O-side chain. The O-side chains are responsible for the development of the diagnostic antibody responses of an animal to brucellosis infection **Halling and Muller (2002).**

The bovine brucellosis control program in Egypt is based on the vaccination with (*Br. abortus* S19 or RB51) and surveillance, movement control within and outside herd. The test and policy slaughter for infected flocks and treatment of meat and milk products had been established.

The aim of this study is detection of annually endemic situation of bovine brucellosis in dairy farms allover 4 years (2008- 2011) after RB51 vaccination, through detection of abortion rate, seroprevalence, Isolation and PCR identification of *brucella species*.

MATERIAL AND METHODS

1-Animals

All over 4 years from 2008 to 2011 a total of 3382 animals located in 4 dairy farms were screened clinically, serologically and bacteriologically for brucellosis to estimate the efficiency of control program in dairy farms. The farms using natural mating by *brucella* free bulls, Cows of different ages and gestation stages, lactating and non- lactating were clinically examined for abortion and breeding troubles including retained placenta, retained placenta with difficult birth, endometritis and repeat breeder. Table (1).

2- Samples:-

a. Serum samples

Blood samples were collected from cows and heifers for serological diagnosis before revaccination, the blood was collected from jugular vein in dry clean sterile tube and serum were separated and preserved at -20 until used.

b. Tissue samples: -

Supra mammary, internal iliac and superficial cervical lymph nodes of adults slaughtered serologically confirmed cows beside fetal fluid, placenta and aborted foeti (liver, lung, spleen and abomasum) samples were collected freshly in ice box and preserved for isolation of *brucella* species.

3- Vaccine and vaccination: -

- **RB51 vaccine** Supplied from CZ Veterinaria, S.L. Aptodo. 16 –Porrino (Pontevedra) 36400 Espana . Batch No. 99001. U.S.A. Brucella abortus strain RB51 vaccine was obtained from Denver, Co. work street 80216 U.S.A. Vet. License No. 188 4950.All the heifer were vaccinated at age from 5-8 months and then before breeding and all the cows annually vaccinated after calving by the recommended dose and route (2 ml s/c injection)

Table (1): Total number of animals investigated against brucellosis in some private dairy farms in Sharkia and Dakhlia Governorates.

Year	Private Farms animals (vaccinated herds)			
	Pregnant cows	Heifer	Male	Total
2008	1030	124	49	**1203**
2009	842	65	36	**943**
2010	538	92	70	**700**
2011	392	76	68	**536**
Total	**2802**	**357**	**223**	**3382**

4- Brucella antigens.

Smooth antigens, Rough antigen, and Brucella abortus antigen, Lipopolysaccharide (rough and smooth antigen):- t it was prepared and supplied kindly from the Department of *Brucella*, Animal Health Research Institute, Dokki, Giza**.** Egypt

5- Brucella primers

Table (2): Oligonucleotide primers used for Brucella DNA amplification.

Primer code	Primer sequence	Product size	Species specificity
Is711 –sp	5' TGCCGATCACTTAGGCCTTTTCCAATCCC '3	498bp	*Br. abortus* (biotype 1,2&3)
Bm - sp	5'AAATCGCCTTGCTGGTCTGA'3	731 bp	Br. melitensis

6- **Indirect Enzyme Linked Immunosorbent Assay: -** was carried according to **Pefanis *et al.*, (1988)** and the test applied according to **Rotz *et al.*, (2002).**

7- **Complement Fixation test: -** Procedure of the CFT was applied according to **Alton et al., (1988).**

8- **Bacteriological examination and typing of Brucella isolates:-**Were according to **(Quinn 1994)**

9- **RCR: -** Was applied according to **(Sambrook, et al 1989** and extraction of DNA was carried out according to **Diaz *et al.*, (1979)**.

10- **Statistical analysis:** Agreement was done according to **Ruppanner *et al.*, (1980),** and Diagnostic studies on sensitivity and specificity were performed according to **OIE (2009).**

RESULTS

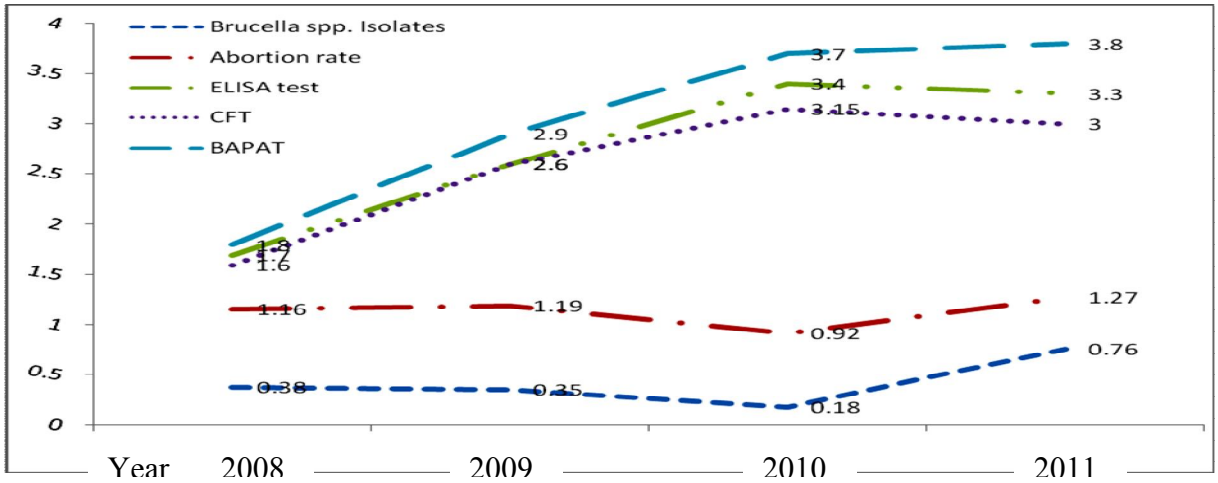
I- Clinical findings of brucellosis:

The clinical findings of brucellosis in dairy farms after application of vaccination program with **RB_{51}** before breeding and after parturition revealed that, the abortion rate was 1.16%, 1.19, 0.92%, 1.27 in 2008, 2009, 2010 and 2011 respectively (**Table 3, Fig 1**).

Table (3): Endemic situation of brucellosis in dairy farms based on serodiagnosis, abortion rate and brucella isolates from 2008 to 2011

Item		**Positive results**	
	Year	**No of** cases/animals	**%**
Screening test BAPAT	2008	19/1030	1.8
	2009	25/842	2.9
	2010	20/538	3.7
	2011	15/392	3.8
Total		**79/2802**	**2.81**
Confirmatory test CFT	2008	16/1030	1.6
	2009	22/842	2.6
	2010	17/538	3.15
	2011	12/392	3
Total		**67/2802**	**2.39**
ELISA test	2008	17/1030	1.7
	2009	22/842	2.6
	2010	18/538	3.4
	2011	13/392	3.3
Total		**70/2802**	**2.49**
Abortion rate	2008	12/1030	1.16
	2009	10/842	1.19
	2010	5/538	0.92
	2011	5/392	1.27
Total		**32/2802**	**1.14**
***Brucella spp.* Isolates**	2008	4/1030	0.38
	2009	3/842	0.35
	2010	1/538	0.18
	2011	3/392	0.76
Total		**11/2802**	**0.39**

Fig (1): Endemic situation of brucellosis in dairy farms based on serodiagnosis, abortion rate and *brucella* isolates from 2008 to 2011



The serological screenings to the animal in area of study revealed the total prevalence rate were 2.81%, 2.39%, and 2.49%, by BAPAT, CFT and ELISA respectively. The annually prevalence showed non-significant difference between years and different serological tests (**Table 3)**

III- Isolation and identification of Brucella spp

Bacteriological examination from available samples (20) collected from aborted fetus allover 4years revealed 11 *Br. melitensis biovar*-3isolates by PCR **(Fig 2)**

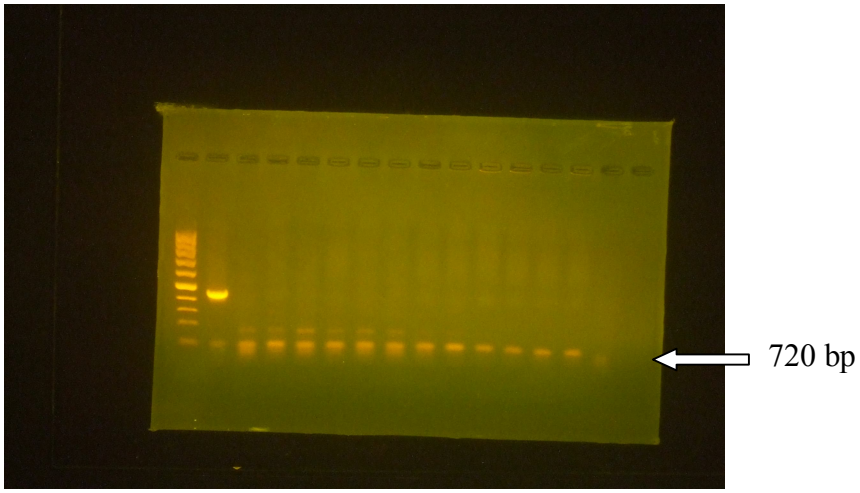


Fig (2): Agarose gel electrophoresis and ethidium bromide staining. Lane M standard DNA marker, lane 1 positive control, lane 2 negative control, and 11 positive isolated samples . The 720-bp PCR product is indicated.

DISCUSSION

Recording of the clinical finding of brucellosis in dairy farms after application of vaccination program with **RB_{51}** before breeding and after parturition revealed that, The abortion rate record was 1.16%, 1.19, 0.92%, 1.27 in 2008, 2009, 2010 and 2011 respectively in dairy farms (**Table 3, Fig 1**). This result was in agree with **Abdel- Hafez, (1996), Kadry et al. (2004) and Radostits et al. (2007**) who revealed that the clinical findings are dependent upon the immune status of the herd and in highly susceptible nonvaccinated pregnant cattle, abortion after the 5th month of pregnancy is the typical feature of the disease in cattle. In recent years, particularly in areas where vaccination is extensively practiced, an insidious form of the disease may develop, which spreads much more slowly and in which abortion is much less common.

The serological screenings to the animal in area of study revealed the total prevalence rate were 2.81%, 2.39%, and 2.49%, by BAPAT, CFT and ELISA respectively. The annually prevalence showed non-significant difference between years and different serological tests (**Table 4 Fig 2)**, in the fact, there about 30 different serological tests for detection of brucellosis **(Alton *et al.,* 1988; and OIE, 2009)**. More over, there is no single test that can correctly identify all infected cases in a single examination. Still, every single test has its own sphere of usefulness. The results of a panel of selected immunoassays were interpreted in parallel rather than in series. Series interpretation means that an animal is considered positive if it is positive to all confirmatory tests applied **(OIE, 2009)** parallel interpretation results in a positive sample if it has a positive result to any of confirmatory test used **(Alton *et al.,*1988).** For every ruminant's species, animals were positive to any of the specific test as CFT and ELISA were considered true positive **(OIE, 2009).**

These results were lower than those reported by **Salem *et al.* (1987), Hamdy (1989)** and **Montasser *et al.*, (1991)** whose recorded 16.1 %, 37.4 %, and 26 %, respectively and this attributed to using of RB_{51} vaccine**,** the heifer calves vaccination at 3, 5, and7 months of age with the strain RB51 vaccine were protected when challenged against infection and abortion during their first pregnancy **Radosits et al. (2007)**. None of the heifers developed antibodies that reacted in the standard agglutination test and in pregnant cattle, SRB51 vaccine when given subcutaneously does not cause placentitis or abortion and the induced humoral and cell-mediated imrnune response does not interfere with the serological diagnosis of field infections **Palmer *et al.,* (1996).** Vaccination with a reduced dosage of SRB51 (reduced dose vaccination) protects adult cattle against abortion or infection caused by exposure to virulent B. abortus during the subsequent pregnancy**.** Revaccination of cows with a reduced dose of

SRB51 in endemic zones does not cause abortion and protects 94 % of animals against field infection but may cause an atypical response to conventional serological tests. Vaccination will markedly reduce the incidence of abortion but the level of infection will not be reduced at a corresponding rate. Even with a widespread vaccination program there will be foci of infection, which are perpetuated indefinitely and few infected cows ever recover from infection completely and should be considered as permanent carriers whether or not abortion occurs. Excretion of the organism in the milk is usually intermittent, is more common during late lactation and can persist for several years **Radostitis (2007).**

The incidence was similar to those obtained by **Kadry *et al.*, (2004)** who stated that the prevalence of brucellosis among cattle, buffaloes, sheep and goats were 1.17 %, 0.6 %, 2.2 % and 0.7 % respectively.

Isolation and identification of *Brucella species* in private farms lymph nodes (nd the isolated bacteria identified by PCR **Fig (3)** this result agrees with ***Alton et al.,* (1988), Kadry *et al.*, (2004)** and **Shannon *et al.,* (2008)** who isolate Brucella species from lymph nodes.

PCR has increasingly been used as a supplementary method in *Brucella species* diagnosis **(Guarino *et al.,* 2001).** 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 **(Ficht *et al.,* 1990).** The author stated that, the Omp2 gene exists as a locus of two nearly homologous repeated copies that differ slightly among Brucella species and biotypes**.**

The previous information were used to design specific primers that amplify a 720 bp fragment, the positive samples taken from farms after vaccination with RB_{51} vaccine, 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 *Br. melitensis* and *Br. abortus*, the test is specific for distinguishing between 2 species **(Ficht *et al.,* 1990).**

Although this study based on strictly RB_{51} vaccination to selected dairy farms the clinical brucellosis is detected and brucellosis were diagnosed serologically and bacteriologically with nearly similar prevalence rate allover 4years which indicate the efficacy of RB_{51} vaccination program in decrease abortion and infection rates but it failed in elimination of brucellosis from this farms so, we need to insure the effectiveness each program components and each one needs to be scientifically sound an accepted by all concerned.

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الملخص العربى

الوضع الوبائي لمرض البروسيلا في مزارع الألبان بعد التحصين باستخدام RB51

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الهدف من هذه الدراسة هو الكشف عن الحالات الوبائية من بروسيلا الأبقار في مزارع الألبان بعد التطعيم RB51 علي مدار أربع سنوات (٢٠٠٨ ـ ٢٠١١). حيث كانت معدلات الإجهاض ١,١٦٪، ١,١٩٪، ٠,٩٢٪، ١,٢٧٪ في عام ٢٠٠٨، و ٢٠٠٩ و ٢٠١٠ و ٢٠١١ على التوالي. في حين أن معدل الإصابة كانت ٢,٨١٪، ٢,٣٩٪، ٢,٤٩٪ باستخدام اختبار BAPAT، CFT وELISA على التوالي. و أظهرت النتائج بأنه يوجد اختلافات كبيرة بين السنوات باستخدام الاختبارات المصلية المختلفة. وخلصت الدراسة إلى أن استخدام تحصين RB51 له فعالية في خفض معدل الإجهاض والإصابة، لكنها فشلت في القضاء على البروسيلا التي تحتاج إلى إعادة تقييم مكونات برنامج مكافحة الحمى المالطية وتحتاج إلى مزيد من الدراسة للوضع الوبائي للبروسيلا .