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Original Research Article

Prevalence of brucellosis in buffaloes and its control measures

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ABSTRACT

Brucellosis is considered an economically important highly contagious and zoonotic bacterial disease of water buffaloes. Control of brucellosis in buffaloes is very important for public health. The efficacy of control program depends on the detection and eradication of infected animals coupled with vaccination and application of biosecurity. This study was carried out to control the brucellosis in buffalo farm in Assuit Governorate, Egypt during the period from April 2015 to August 2016. Out of 620 unvaccinated buffaloes, 87 (14.03%) aborted. Moreover, 90/620(14.51%), 82/620(13.22%), 82/620(13.22%), and 80/620 (12.9%) buffaloes were serologically positive by BAPA, RBPT, m SAT and Riv.T, respectively. Three isolates were differentiated as *Brucella melitensis*, biovar 3, one strain isolated from one vaginal swap out of 10 Riv.T. positive recently aborted buffaloes (10%) and two strains were isolated out of ten milk samples of Riv.T. positive buffaloes (20%). Eighty serological positive buffaloes to Riv.T were culled from the herd, while 60 serological negative heifers were vaccinated by *Brucella abortus* S 19 vaccine, with a dose of 3-8×109 cfu/5ml and monitored for serological titer for 240 days. After 6 months of vaccination, the number of serologically positive calves declined marginally to 50 (83.33%), 40 (66.67%), 50 (83.33%), 0 (0%), 40 (66.67%) and 0 (0%) by BAPA, RBPT, mSAT, CFT, iELISA and cELISA, respectively. Three successive serological tests every three weeks were done by screening tests, BAPA and RBPT and confirmed by Riv.T. At the end of the control program, all examined buffaloes were serologically negative. Application of biosecurity in the farm was applied by the sanitary disposal of aborted material and application of proper disinfectants at its recommended work strength and contact time.

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1. Introduction

Brucellosis is a highly contagious bacterial disease primarily affects domestic and wild animals and has both economic and public health implications. It is economically important as it causes financial losses due to abortions, sterility, decreased milk production, veterinary fees and costs of replacement animals (Radostits et al., 2000; Madhavaprasad et al., 2014). In humans, it is characterized by headaches, joint pain, undulating fever and general body malaise (Bouley et al., 2012), therefore highlighting the importance of its control (OIE, 2009).

Control of brucellosis presents considerable difficulties due to its wide distribution in many countries of the world, wide host range, significant numbers of carrier cases and latently infected animals as well as difficulties of diagnosis. This disease is very dangerous for humans, which often leads to disability and sometimes to permanent disability (Albertyan, 2009). Genus *Brucella* merges nine different species: *B. melitensis*, *B.abortus*, *B.suis*, *B.canis*, *B.neotomae*, *B.ovis*, *B.ceti* (the causative agent of cetacean's brucellosis), *B.pinnipedialis* (the causative agent of pinnipeds brucellosis) and *B.microti* (the causative agent of gray voles brucellosis) (Zheludkov and Tsirelson, 2010; Sklyarov et al., 2011). Nowadays, genus *Brucella* includes more than10 species (Godfroid et al., 2011; Mailles et al., 2012). The most of them are pathogenic for humans.

Bovine brucellosis is one of the most important infectious diseases affecting bovine (Corbel, 1997), occurring worldwide except where veterinary efforts have been able to eradicate it (Seleem et al., 2010). Brucellosis in buffaloes is one of the main reproductive diseases capable of causing abortion storms in the breeding season during the last third of pregnancy, retention of the fetal membranes, stillbirths and reduction in milk yield resulting in great economic losses (Neta et al., 2010).

Strategies for control and eradication of bovine brucellosis are currently based on identification of animals, restriction of animal movement and the early detection and removal of infected animals using different diagnostic tests, usually Rose Bengal test (RBT) and Buffered Acidified Plate Antigen Test (BAPA) as a qualitative presumptive screening tests, micro standard agglutination test (mSAT) as a quantitative screening test (Alton et al., 1988), Complement Fixation Test (CFT) as a Quantitative Confirmatory Test (OIE, 2009) and/or Rivanol Test (Riv.T.) as a semi-quantitative quick American confirmatory test within twelve minutes (Alton et al.,1988). In addition, usually at the first stages of control programs, when the prevalence levels are high, vaccination is carried out to avoid dissemination of the causative agent. The most widely used vaccine for the prevention of brucellosis in cattle is the *Brucella abortus* S19 vaccine, (Nicoletti, 1990; Smits, 2013).

The success in eradicating brucellosis in animals is largely dependent on the quality of the veterinary services and administrative organizations involved. The present study describes the different strategies that could be applied to either the control or eradication of brucellosis in buffaloes in a buffalo farm in Assuit Governorate in Egypt.

2. Materials and methods

2.1. Study area

The current research was carried out in governmental farm located at Beni-Sanad Assiut governorate, Egypt (from April 2015 to August 2016). In the first stage, a transverse epidemiological study was carried out to identify animals naturally infected with brucellosis.

2.2. Serological diagnosis

A cross-sectional study was conducted to determine the occurrence of brucellosis in the examined herd. Approximately 7-10 mL of blood was collected from jugular vein using plain vacutainer tubes and needles. Individual tubes were identified using numbers to indicate their location and source. The tubes were left tilted overnight at room temperature to allow clotting. The sera were separated from the clot (unretract blood centrifuged) by siphoning into sterile test tubes. Serum samples were transported in ice-box to the *Brucella* Department, Animal Health Research Institute, Dokki-Giza and stored at -20ºC. The RBT was conducted as previously described (OIE, 2009) and was used to screen sera for anti-*Brucella* antibodies. The buffered acidified plate test, mSAT and Riv T. was carried out using standard techniques (Alton et al., 1988).

2.3. Antigens used for serological tests

A smooth white colored antigen of *B. abortus* strain 99 of SAT and BAPA test, RBPT and Riv. T. were obtained from the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

2.4. *Brucella abortus* concentrate for CFT

It is the USDA standard tube test concentrate (4.5% *B.abortus* biotype1strain 1119-3 cells in phenol saline final pH 6.8).

2.5. Reagents of CFT

Complement and hemolysin were prepared in the department of brucellosis, AHRI, Dokki, Giza. Sheep RBCs were obtained from healthy brucellosis free Ram. Veronal buffer was prepared according to Alton et al. (1988).

2.6. ELISA kits

Indirect ELISA kit: Boehringer Inglheim. Sevanova.Box 1545, se-751-45 Uppsala, Sweden. cELISA Boehringer Inglheim .Svanova, Box 1545, se,-751-45 Uppsala Sweden Kit. Batch number p-00094 Expiry date 13-11-2016. The cELISA was done according to the manufacturer's instructions and essentially as described elsewhere (Muma et al., 2006; Matope et al., 2010). Only positive animals for RBT and cELISA were classified as *Brucella* seropositive.

2.7. Control strategies

Control strategies implemented throughout this study include several special measures (a) restricting movement of breeding animals in these areas; (b) an increase in routine testing, from two to four annual serological tests; (c) compulsory reporting of abortions in animals; (d) segregation and compulsory slaughter of test reactors within 15 days; (e) in the case of slaughter of infected animals, disinfection under official supervision and quarantine of infected facilities (including pastures, stables) for at least 90 days after removal of test positive animals; and (f) young serologically negative heifers and replacement heifers (3–6 months of age) were compulsory vaccinated with *B. abortus* S19 vaccine, which was obtained from Coopers Animal Health Inc., Kansas City, USA. The dose used to vaccinate heifers was $3–8\times10^9$ cfu/5ml injected subcutaneously in the side of the neck.

2.8. Assessment of responses to immunizations

Blood samples were weekly collected from each of vaccinated heifers during the first month and then Monthly till day 200 post vaccination (p.v.). Serum samples from vaccinated heifers were examined for *B. abortus* antibodies by the BAPA, RBPT, SAT and CFT performed as described by Alton et al. (1988). Collected samples were examined also by iELISA and cELISA performed as described by Wright and Nielsen (1988). The SAT titers were expressed in international units per ml (I.u. /ml). Titers > 100 were considered positive for vaccinated buffalo and those below that were negative. During the course of this study, vaccinated animals were observed for principal manifestations of brucellosis like abortion, stillbirth, retention of placenta and infertility.

2.9. Bacteriological study

To determine the possible involvement of S19 vaccination strains when reproductive failures were reported, vaginal swabs from aborted buffaloes and samples from abortions were collected for isolation of the etiological agent. In addition, prescabular and supra-mammary lymph nodes from a proportion of seropositive animals were sampled in the abattoir. All samples were processed according to the INRA "Manual for the Brucellosis Laboratory" (Alton et al., 1988). *Brucella* agar medium was used for isolation, and positive cultures were identified as *Brucella* spp. based on colony and bacterial morphology, staining characteristics and reaction against positive polyclonal serum reaction against monospecific antisera (A, M and R), carbon dioxide requirement, H_2S production and growth on different concentration of thionine and fuchsine dies.

3. Results

Table 1. Serological profile of buffalo farm infected with brucellosis.

No. of animals	Serological tests								
	BAPA		RBPT		mSAT		RIVT		
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
620	90	530	82	538	78	4	538	80	540
Number	90	530	82	538	82		538	80	540
Percent	14.51	85.49	13.22	86.78	13.22		86.78	12.9	86.1

Table 2. Agreement and disagreement between different serological tests in buffalo farm infected with brucellosis.

Serological test		BAPA		RBPT		SAT		RIV.T	
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
BAPA	**+ve**			82	8	82	8	80	10
	-ve			0	530	0	530	0	530
RBPT	**+ve**	82	0			82	0	80	2
	-ve	8	530			0	538	0	538
SAT	**+ve**	82	0	82	0			80	2
	-ve	8	530	0	538			0	538
RIV.T	**+ve**	80	0	80	0	80	2		
	-ve	10	530	2	538	2	538		

Table 3. Epidemiological study in a buffalo farm.

Item		Private buffalo farm
Total number of animal		620
Number of replacement bulls		5
True prevalence		12.9
Abortion rate	**Number**	87
	percentage	14.03
Number of reactor animals		80
Number of *Brucella* isolates		3

Table 4. The overall seroprevalence of brucellosis in buffaloes based on Rivanol Test.

Risk factors	No. tested	Infected (%)	Healthy (%)
Age of animals (month)	110	0(0)	110(100)
Age of animals (year)			
2 - 3.5	300	33(11)	267(89)
3.6 – 4	120	25(20.83)	95(79.17)
>4	90	22(24.44)	68(75.56)
Parity (No.)			
Up to 2	30	3(10)	27(90)
2 – 4	80	10(12.5)	70(87.5)
>4	100	16(16)	84(84)
Previous history of abortion			
Yes	87	80(91.95)	7(8.05)
No	143	0(0)	143(100)
Reproductive status			
Heifer	110	0(0)	110(100)
Pregnant	100	0(0)	100(100)
Lactating	230	80(34.78)	150(65.22)
Dry	180	0(0)	180(100)

Table 5. The profile of heifers vaccinated with *Brucella abortus* S19vaccine.

Days post. Vacc.	No. of animals	Serological tests												
		BAPAT		RBPT		mSAT			CFT		iELISA		cELISA	
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
0	**60**	-	-	-	-	-	-	-	-	-	-	-	-	-
15	**60**	60	-	60	-	54	-	6	24	36	60	-	18	42
30	**60**	60	-	60	-	60	-	-	24	36	60	-	-	60
60	**60**	60	-	60	-	60	-	-	18	42	60	-	-	60
90	**60**	60	-	60	-	60	-	-	-	60	60	-	-	60
120	**60**	60	-	60	-	60			-	60	60	-	-	60
150	**60**	60	-	60	-	60			-	60	60	-	-	60
180	**60**	50	10	40	20	30	10	20	-	60	40	20	-	60
210	**60**	-	60	-	60	10	40	10	-	60	-	60	-	60
240	**60**	-	60		60	-	50	10	-	60	-	60	-	60

BAPAT: Buffered acidified plate antigen test.
RBPT: Rose Bengal Plate Test.
mSAT: Microplate Serum Agglutination Test.
CFT: Complement Fixation Test.
cELISA: Competitive ELISA.
iELISA: Indirect ELISA

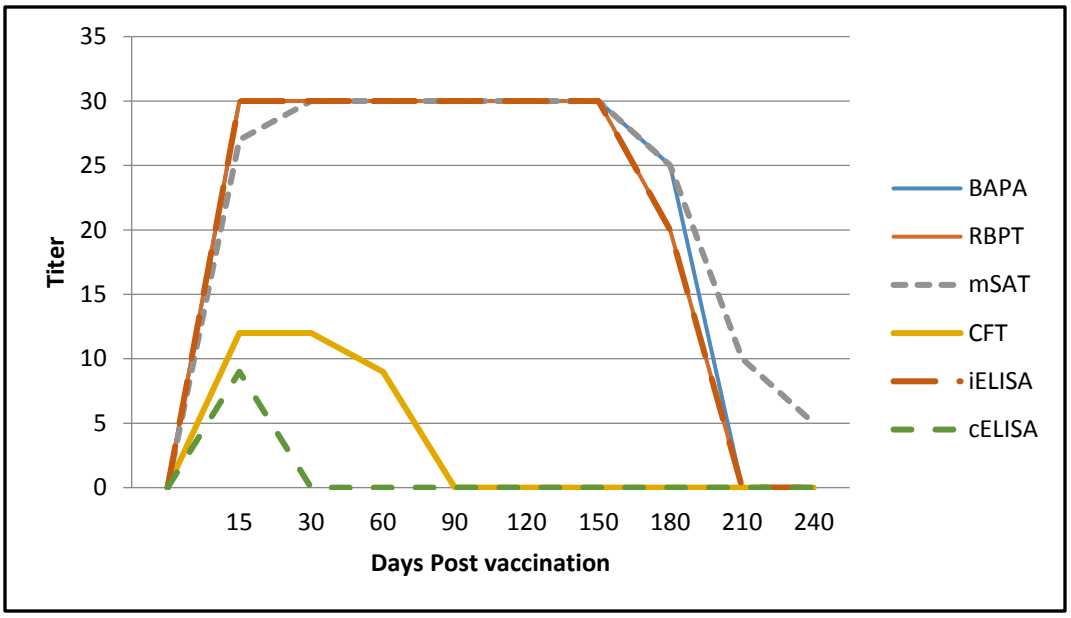

Fig. 1. Post vaccination elicited titer by different serological tests.

Table 6. Examination of the farm by three serological tests of brucellosis with three weeks intervals between each test after culling of infected buffaloes of different reproductive status.

		BAPAT			RBPT			Riv.T		
Age of animals (year)	No. of animals	1st exam	2nd exam	3rd exam	1st exam	2nd exam	3rd exam	1st exam	2nd exam	3rd exam
1	100	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	247	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2 – 4	95	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
>4	98	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Total number of examined buffaloes 540										

BAPAT: Buffered acidified plate antigen test.
RBPT: Rose Bengal Plate Test.

4. Discussion

Brucellosis is an important zoonosis and serological surveillance is essential to its control (Raghunatha et al., 2014). Although the eradication programs have been established by vaccination and test and slaughter of the *Brucella* infected animals, the disease still remains as a major zoonosis all over the world (Kakoma et al., 2003; Madhavaprasad et al., 2014). From April 2015 to August 2016, the prevalence of the disease in buffaloes was 12.9 (Tables 1-3). Such prevalence was less than that reported by Islam et al. (2013) (13.33%) in buffaloes, and more than that reported by Rahman et al. (2012) (8.33%) in buffaloes in Mymensingh district.

The lower positive incidence of RIV.T than RBRT and BAPAT may be due to the precipitating activities of Rivanol solution of the IgM so the test only detect IgG2 immunoglobulin as recorded by Pietz and Gowart (1980). The specificity of RIVT was reported to be high in diagnosis of brucellosis in the examined farm animals which agreed with the results reported by different authors (Nicoletti, 1992; El-Enbawy et al., 1995). Variation in the incidence of infection is related to the course of the diseases,

locality, rate of exposure, reproductive status, sex, improvements in the diagnostic techniques and vaccination strategies (Ghazi et al., 2006). It has been reported that the genetic variation within the host may play a part in the resistance to brucellosis (Silva et al., 2013).

It has been found that, out of 87(14.03%) aborted buffaloes, 3 (3.45 %) were positive for culture and isolates were identified as *B. melitensis* biovar 3 (Table 3). One strain was isolated from one vaginal swap out of ten of recent aborted buffaloes positive to RIV.T. (10%) and two strains were isolated from milk samples (20%) out of ten buffalo positive to RIV.T. A higher rate of isolation (3/32) of *B. melilensis* from the supramammary lymph node obtained from infected buffaloes was reported by Ahmed et al. (2010). Meanwhile, the prevalence of brucellosis was higher in animals with previous abortion record in buffaloes, than that with no abortion similarly that recorded by Rahman et al. (2012). In the current study, buffaloes aged more than 4 years had higher prevalence (24.44%) than other age groups, and the animals that experienced abortion showed the highest prevalence of brucellosis. Similar observations were also recorded by Vikrant et al. (2006) and Islam et al. (2013). Age-wise prevalence has also been studied by Abubakar et al. (2010) who showed that the incidence of brucellosis increased with age, and the incidence is high in sexually mature animals.

Execution of control program was started with control of animal movement and identification of the animals then screening of animals, segregation of positive population followed by cleaning, disinfection and decontamination of premises particularly of calving pens and area surrounding the pen to reduce antigenic load at the farm. Subsequently, negative heifers 3-8 months (*n*= 60) except those aged below 4 months (*n*=5) and males (*n*=45), were vaccinated by reduced dose of *B. abortus* S19 vaccine. Strain 19 is the most commonly used in vaccination program against bovine brucellosis in Egypt and all over the world. The main advantage of S19 is its considerable humeral and cellular protection against brucellosis even when we use it at a reduced dose. Yet its main disadvantage is the production of smooth antibodies which interfere with the diagnosis of disease using conventional serological tests (Alton et al., 1984; Crawford et al., 1991). In animals vaccinated with S19, IgM, IgG1 and IgG2 (humeral antibodies) are produced. After six months, IgG2 has usually disappeared, but very low levels of IgM and IgG1 may be present, often in concentrations, which are too low to be detected by the CFT. In infected animals, higher levels of IgG1 are usually present and these are detected by the CFT.

Antibody response to vaccination was detected by the increase in the positivity percent in each group of animals from two weeks up to 8 months, which was monitored by BAPA, RBPT, mSAT, CFT, iELISA and cELISA (Table 5). The 60 heifers aged 4 to 8 months, vaccinated subcutaneously with standard dose of S19 vaccine, were positive in BAPA, RBPT, mSAT, CFT, iELISA and cELISA after 2 weeks of vaccination. After 6 months of vaccination the number of serologically positive calves declined marginally to 50 (83.33%) 40 (66.67%), 50 (83.33%), 0 (0%), 40 (66.67%) and 0 (0%) by BAPA, RBPT, mSAT, CFT, iELISA and cELISA respectively. Brucellosis is the biggest threat to the dairy farming in Egypt as it causes tremendous economic losses once enters in animals at the farm, so the owner should be compensated these losses enough to be honest with the authorities for condemnation of the positive animals. Similar report recorded by Chand et al. (2013) who revealed that 30 (90.90%) calves aged 4-8 months, vaccinated subcutaneously with standard dose of S19 vaccine, were positive in RBT after 1 month of vaccination.

After 3 months of vaccination the number of RBT positive calves declined marginally to 24 (72.72%).Furthermore, more than 90% of heifers vaccinated with S19 were classified negative by classical serological tests (CFT) at 16 weeks post-vaccination, while they were still classified positive by iELISA as recorded by Saegerman et al. (1999). cELISA was used as more sensitive and specific alternative to conventional test such as RBT, which is unable to distinguish between *B. abortus* strain 19 vaccinated animals and naturally infected animals (Gall and Nielsen, 2004). Vaccination induces antibody thought to be of lower affinity due to a short exposure time to the antigen because it is eliminated by the immune system. Alternatively, antibody produced in response to natural infection is of higher affinity because the antigen is not removed as quickly by the immune system; therefore, persist for much longer period (Macmillan, 1990). Thus, cELISA was developed to overcome this problem. It is nearly distinguishing vaccinated animals or

animals infected with cross-reacting organisms from naturally infected animals, thereby reducing the number of false-positive reactions (Gall and Nielsen, 2004), so it is a highly specific and sensitive diagnostic assay since it directly detects antibody and has minimal or no false positive reactions of agglutination test and its results provide an epidemiological tool for investigating the infective status of flocks (Mustafa et al., 2012).

The Governmental project is compelled to bear heavy economic losses because effective control program which included vaccination of young animals is not in place. Moreover, farmers/ dairymen are unaware of brucellosis and they came to know about the disease only after losses had occurred. Initial efforts to contain and control brucellosis at the farm by segregation of positive animals were not successful as abortions continued in subsequent months. The most likely reason appeared to be the highly contaminated environment which remained source of infection to negative but unvaccinated population. In (2015), a strategy of testing and segregation of sero-positive animals, decontamination of farm premises and vaccination of negative female animals was adopted. The number of infected buffaloes in the far was impossible to institute management procedures for the control of brucellosis (Radostitis et al., 2000). However, post vaccination antibody titers persisted for quite a long period which interfered in subsequent testing. The problem of persistent antibody titers was resolved by using CFT and cELISA for testing heifers after vaccination these results agree with the results of El-Bauomy et al. (2014).

Accordingly, to control brucellosis in the farm in shortest possible time, segregation of positive animals coupled with vaccination of negative young female animals is needed. The young negative males (n=45) were fattened to compensate some losses. The other adult male and non-pregnant female buffaloes were examined by three presumptive tests with three weeks intervals and gave negative results (Table 6). The strategy presented in this study to control brucellosis on an endemically infected Governmental buffalo farm could serve as a model for private animal farms elsewhere in the country.

5. Conclusion

The present study reveals that bovine brucellosis is a problem of concern in buffaloes. Several factors are related to the occurrence and prevalence of *Brucella* infection including abortion, poor disposal of aborted material, vaccination, veterinary services, and lack of knowledge on the transmission of brucellosis in buffaloes. A combination of several serological tests including presumptive tests (BAPAT and RBPT) should be applied to exclude negative cases which are usually of high sensitivity, followed by a confirmatory tests of high specificity such as (RIV .T) and all reactors should be removed from the herd. *Brucella melitensis* (biovar 3) is the dominant strain in Egypt. Parities number and history of abortion in a herd shown to be the major factors associated with finding positively testing animals in a herd. A control program for brucellosis in buffalo farm should be based on routine testing and slaughter of seropositive buffaloes and vaccination of all female animals accompanied by application of hygienic measures such as restriction of animal movement and improved farm sanitation to reduce the further spread of the disease.

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