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Evaluation of field application of *Brucella abortus* (Abortox) vaccine in buffaloes and cattle

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ABSTRACT

The field application of 45/20 rough killed *Brucella abortus* (Abortox) vaccine conferred a significant degree of protection to buffaloes and cattle. Abortox vaccine is the first trial of vaccination on Egyptian buffaloes. Two lines of treatment were adopted. The first one was based on test and slaughter of the positive reactors, and the second was the isolation of the positive and suspicious animals after vaccination and, repeated serological testing of them till they became serologically negative or were slaughtered.

For many years attempts to control brucellosis in domestic animals have been based on the serological identification and slaughter of the reactors (Stableforth and Galloway, 1959). However, this method is very costly. Therefore, vaccination has achieved great importance for the control of this disease (Guilloteau, 1972). The live vaccines give a good protective effect, but produce a persisting antibody titre for a long time which may interfere with the serological diagnosis, as it would be difficult to distinguish serologically between infected and vaccinated animals. To overcome this problem a rough vaccine that gives antibodies unagglutinable with the commonly used smooth antigen was introduced by McEwen and Priestly (1938). Such a vaccine together with an adjuvant was used in cattle and recommended by other authors (Sutherland *et al.*, 1981; Corner *et al.*, 1983). No available literature could be found about the application of Abortox in buffaloes. The aim of this study was to evaluate the field application of the rough vaccine (Abortox) both in buffaloes and cattle in correlation with a comparative study between the methods of controlling brucellosis.

MATERIALS AND METHODS

In this investigation 3,533 animals (3,433 cattle and 100 buffaloes) were used. These animals were located in 4 farms. The first farm included 100 buffaloes purchased for Mahalet Mousa Farm. These animals were not examined serologically before vaccination, i.e. they were vaccinated under unknown circumstances. After 4 months of vaccination their sera were subjected to tube agglutination test (TAT), mercaptoethanol test (MET) and complement-fixation test (CFT) (Alton and Jones, 1967; Alton *et al.*, 1975). These animals were vaccinated with 2 doses of Abortox vaccine 2 months apart. The negatives and reactors were isolated in separate places. Blood samples of all animals were examined at 4, 6, 7, 8, 9 and 10 months after the last dose of vaccination. Titres of 1/50 and 1/80 were considered to be positive, while 1/20 was considered as suspicious (Alton *et al.*,

1975).

The second farm located in North Tahrir Province included 1,000 Friesian cows. Their sera were collected and examined with TAT and CFT. They gave completely negative results before vaccination. These animals were vaccinated with 2 doses of Abortox at 8-week intervals. After 6 months from the second dose of vaccination these animals were examined serologically, and the positive and suspicious reactors were slaughtered. A booster dose was given after 8 months when all the animals were serologically negative. After 1 and 2 months, the animals were examined and the reactors eliminated. During the next 4 months a new flock of sheep (740) was purchased and left to share the pasture with the cows. Abortion occurred in cattle and sheep. Therefore sera, milk and aborted foeti were collected and examined bacteriologically and serologically. The farm was put under quarantine measures and examined every 2 weeks.

The third farm included 1,927 Friesian cows with a history of *Brucella* infection. The animals were vaccinated with Abortox vaccine in 2 doses, 2 months apart and their sera were examined before vaccination, 2 months after the first dose of vaccination, and then 1, 2, 3, 4 and 5 months after the second dose of vaccination. The positive reactors were slaughtered while the suspicious reactors were isolated from the serologically negative animals, examined till they became negative serologically and then added to the herd.

The fourth farm included 506 Friesian cows which were vaccinated with Abortox vaccine in 2 doses 1 month apart. Sera were collected and examined by TAT and Rose Bengal test (RBT) (Morgan *et al.*, 1969) before vaccination 1 and 6 months as well as 1 year after vaccination. The animals were given a booster dose, and then examined at 1, 2, 3, 4, 5, 6, 7 and 8 months later. The positive or suspicious reactors were isolated in separate places and examined till they became serologically negative. All negative animals were added to the herd while the positive ones were slaughtered.

RESULTS

The results of antibody titre among sera of the buffaloes (first farm) revealed 39 positive reactors (with maximum titre 1/80) and 18 suspicious reactions (1/20) with TAT. After 6 months of vaccination only 24 serum samples were positive (1/80) and 13 gave suspicious reaction; the negative samples were 63. On the other hand, the MET revealed 16 positive reaction, half of them only gave titre 1/80 and 7 gave titre 1/20. After 7 months, these 100 sera samples were subjected to TAT, MET and CFT. TAT revealed 2 sera with positive reaction of 1/80 titre; the other 8 were suspicious. ME gave 4 samples with a titre of 1/40 and 11 had suspicious reaction. But the CFT revealed 5 cases with titre of 1/40 and 10 cases with 1/20. At 8 months, all sera collected from buffaloes were negative with TAT and MET while 2 cases gave 1/40 and 3 cases 1/20. After 9 and 10 months the farm became serologically negative (Table 1).

In the second farm, all the 1,000 cows were completely negative before any vaccination. Examination after 6 months from the second dose of vaccine revealed 1 positive case and 4 suspicious cases, all of which were slaughtered. A total of 995 animals were revaccinated with 1 dose (booster) of the same vaccine; 1 month later serum was collected from all the animals (995) and examined with TAT and CFT which revealed 10 positive and 5 suspicious cases. All the reactors were slaughtered. The remaining 980

animals were serologically negative. Two months after booster dose 4 positive cases were detected and they were slaughtered. During this time 740 sheep were introduced into the farm; they and shared the pasture with cows (976 cows). This was followed by the occurrence of a storm of abortion in ewes and cows. Out of the 740 sheep, 450 were positive and 81 suspicious; 26 cows were positive serologically. *Brucella melitensis* could be isolated from slaughtered animals, milk of positive cases and foeti of aborted animals. At 8 months from exposure to natural infection, 56 cows were positive and 130 were suspicious. All the reactors were slaughtered and the farm was put under quarantine measures with examination every 2 weeks till 3 successive negative tests were obtained.

These results indicated that the natural infection existed in this farm which is considered as a challenge. The animals vaccinated with Abortox gave some degree of protection against *Br. melitensis* infection (Table 2).

The control measures on farm 3 were adopted to slaughter the positive and isolate the suspicious animals. During examination 819 animals were serologically negative; 169 suspicious cases were kept in a separate place (Table 3). Only 59 cases became positive. These were slaughtered. The suspicious animals were isolated and re-examined. There was a reduction of positive cases and only 9 cases persisted after 4 months. These positive cases were slaughtered and the farm became serologically negative.

The examination of the fourth farm with TAT, MET and RBT revealed post-vaccinal reaction after booster dose which gave 4 cases suspicious after 6 months and still positive till 8 months (Table 4). The 4 cases were slaughtered and the farm became serologically negative.

DISCUSSION

The vaccination of buffaloes (Farm 1) with Abortox vaccine was the first trial in Egyptian buffaloes. It is difficult, however, to discuss the findings in buffaloes as no references dealing with application of Abortox vaccine in buffaloes could be found in the available literature.

The field application of 45/20 killed *Brucella abortus* conferred a significant degree of protection to buffaloes and cattle. This is in agreement with the results obtained by Roerink (1966), Morgan *et al.* (1968), Dhennin (1973) and Alton *et al.* (1983). TAT and MET tended to regress over 7 months or so to negative levels so that animals with CF antibody titres would be allowed to remain up to about 8 months. Cunningham and O'Connor (1971) and Corner *et al.* (1983) found that the 45/20 vaccine stimulated the appearance of CF antibodies in cattle that were CFT negative at the time of vaccination.

The positive and suspicious titres disappeared gradually and the animals became in a short period free from agglutinating antibodies. This indicated that the animals that showed titres were not in the incubation stage as interpreted by Roerink (1967). This transient titre was most probably evoked by the 45/20 vaccine. This opinion has been expressed by Cunningham and O'Railly (1968) who concluded that 45/50 vaccine may show evidence of a low content of agglutinable brucella (smooth) and that the agglutinin response was quite low and transient also in adult animals. On the other hand, the natural infection is considered a means of challenge in the vaccinated animals with 45/20 which gave a slight protection against *Br. melitensis* infection. The occurrence of abortion storm in the farm was probably due to the introduction of sheep and decrease of resistance of

Table 1. Antibody titres among sera of buffaloes (first farm) vaccinated with K 45/20 Abortox vaccine

Time of examination after vaccination (months)	No. of animals	Serological tests																	
		Tube agglutination						Mercaptoethanol						Complement fixation					
		1/20	1/40	1/80	No.	%	Neg.	1/20	1/40	1/80	No.	%	Neg.	1/20	1/40	1/80	No.	%	Neg.
4	100	18	13	26	57	57	43	Not applied						Not applied					
6	100	13	11	13	37	37	63	7	8	8	23	23	77	Not applied					
7	100	8	8	2	18	18	82	11	4	-	15	-15	85	10	5	15		15	85
8	100	-	-	-	-	-	100	-		-	-	-	-100	3	2	-	5	5	95
9	100	-	-	-	-	-	100	-	-	-	-		-100	-	-	-	-	-	100
10	100	-	-	-	-	-	100	-	-	-	-	-	100	-	-		-	-	100

Table 2. Antibody titres among sera of animals (1,000 cows) in the second farm

Time of examination after vaccination (months)	Animals	No. of animals	Reactors detected by TAT & CFT				Negative	Remarks
			positive		suspicious			
			No.	%	No.	%		
Before vaccination	Cows	1000	-	-	-	-	1000	
6 months post 2nd v.	Cows	1000	1	0.1	4	0.4	995	Positive & suspicious slaughtered
8 months post 2nd v.	Cows	995	-		-	-	995	
Revaccination 1 year booster		Introduced 740 sheep to the farm.						
month after booster	Cows	995	10	1.05	5	0.5	980	Positive & suspicious slaughtered &
	Sheep	740	450	60.8	81	10.9	209	*Br.melitensis* was isolated.
2 months after booster	Cows	980	4	0.4	-	-	976	Positive were slaughtered
3-8 months after booster	Cows	980	4	0.4	-	-	950	Positive were slaughtered
9 months after booster	Cows	950	56	5.89	130	13.68	764	Positive and suspicious slaughtered

The farm put under quarantine measures and examined every 2 weeks; positive and suspicious were slaughtered till the farms became serologically negative 3 successive times.

Table 3. Antibody titres among sera of animals (1927) in the third farm

Time of examination	No.of animals	Reactors detected by TAT & CFT				Negative	Remarks
		Positive		Suspicious			
		No.	%	No.	%		
Before vaccination 1st day	1927	691	35.8	82	4.3	1154	The positive were slaughtered & suspicious isolated
Before revaccination	1236	248	20.0	169	13.7	819	The positive were slaughtered & suspicious isolated
1 month from second dose	819	-	-	-	-	819	The positive were slaughtered & suspicious isolated
(Primary vaccination)	169	59	34.9	31	18.3	79	
2 months from 2nd dose	819	-	-	-	-	819	The positive were slaughtered & suspicious isolated
	110	22	20.0	19	17.5	69	
3 months from 2nd dose	819	-	-	-	-	819	The positive were slaughtered & suspicious isolated
	88	22	2.3	2.9	10.2	77	
4 months from 2nd dose	819	-	-	-	-	819	The positive were slaughtered & suspicious isolated
	86	-	-	9	10.5	77	Suspicious cases were slaughtered
5 months from 2nd dose	819	-	-	-	-	819	
	77	-	-	-	-	819	
6 months from 2nd dose	896	-	-	-	-	896	

Table 4. Antibody titres among sera of animals in the fourth farm (506 cows).

Time of examination post vaccination	Dose of vaccination	No.of animals	Serological tests														
			Tube agglutination						Mercaptoethanol						Rose Bengal		
			1/20	1/40	1/80	No.	%	Neg.	1/20	1/40	1/80	No.	%	Neg..	No.	%	Neg.
Before vaccination	1st	506	-	-	-	-	-	506	-	-	-	-	-	506	-	-	506
One month Pv	2nd	506	-	-	-	-	-	506	-	-	-	-	-	506	-	-	506
6 month Pv		506	-	-	-	-	-	506	-	-	-	-	-	506	-	-	506
1 year Pv	Booster	506	-	-	-	-	-	506	-	-	-	-	-	506	-	-	506
1 month after booster		506	11	8	1	20	33.9	486	6	2	1	9	1.6	497	22	4.1	484
2 months after booster		506	17	7	11	35	6.9	471	12	5	1	18	3.5	488	35	6.9	471
3 months after booster		506	23	5	1	29	5.7	477	6	4	-	10	1.9	496	29	5.7	477
4 months after booster		506	32	5	-	37	7.7	469	5	3	-	8	1.5	498	19	3.7	487
5 months after booster		506	21	1	-	22	4.3	484	-	4	-	4	0.8	502	22	4.3	484
6 months after booster		506	-	4	-	4	0.8	502	-	4	-	4	0.8	502	4	0.8	502
7 months after booster		506	-	4	-	4	0.8	502	-	4	-	0.8	0.8	502	4	0.8	502
8 months after booster		506	-	4	-	4	0.8	502	-	4	-	4	0.8	502	4	0.8	502
9 months after booster		506	-	-	-	-	-	502	-	-	-	-	-	502	-	-	502

4 animals were slaughtered and the herd became free.

the vaccinated animals, or the exposure of the vaccinated animals to high dose of the virulent *Br. melitensis*, or difference in antigenicity between 45/20 and *Br. melitensis*. Ray (1975) observed that 45/20 vaccine did not show adequate resistance against exposure to virulent brucella organisms. These results are not in agreement with those of McKeen (1975) who found that animals vaccinated with Abortox and Duphavac vaccines produced a high level of protection against challenge with *Brucella abortus* 544 as also indicated by Morgan *et al.* (1968) and Sutherland *et al.* (1981). The herd has a history of brucellosis (Farm 3). When vaccinated with Abortox vaccine the herd gave a reduction of the positive cases. However 9 were still positive and they were slaughtered. This indicated that the vaccination may have a diagnostic means particularly after the first vaccination. This is because infected animals show very high titres after the first vaccination. With the aid of these high titres, the infected cattle can be detected rapidly and eliminated by slaughtering, thus quickly reducing the risk of infection (Roerink, 1967).

Two lines of treatment were adopted in this work. The first was based on test and slaughter of the positive reactors and repetition of the monthly test (or every 21 days), till all the animals gave negative results in 3 successive negative examinations with 2 months interval between every examination. This was recommended by Stableforth and Galloway (1959). However, this is sometimes economically unbearable (Guilloteau, 1972). The second was based on the examination and segregation of the positive and doubtful cases and vaccination of the negative ones with 45/20 rough killed *Brucella abortus* vaccine. Repeat the test and segregate the positive ones before the second dose of primary vaccination and then repeat the examination for 4 months successively by getting rid of the persistent reactors until the farm becomes negative completely.

This was based on the observation of Cunningham and O'Railly (1968) and Lannelli and Diaz (1977) who attributed the presence of agglutinins after vaccination with 45/20 vaccine to the presence of small numbers of smooth brucella in the rough vaccine or to common core antigen. On the other hand, Corbel and Bracewell (1976) mentioned that the appearance of smooth antibodies may be due to a close antigenic relationship between the rough and smooth strains of *Brucella abortus*.

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