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COMPARISON OF VARIOUS SEROLOGICAL MILK TESTS FOR SCREENING OF OVINE BRUCELLOSIS

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UMMARY

total of 689 milk samples, collected from ewes different stages of lactation, was examined bac-riologically and serologically for detection of rucella infection among sheep flocks. Bacterio-gical examination was carried out using the or-inary isolation and identification methods for rucella organisms. Serological examination was ased upon three tests specific for milk, namely, bortus Bang Ring Test (ABRT), Whey Aggluti-ation Test (WAT), and Whey Antiglobulin Coomb's" Test (WCT). Comparative evaluation f the different employed methods indicated that e ABRT and WGT were of similar sensitivity nd both tests were more specific than the WAT. . *melitensis* biotypes 2 and 3 were recovered om the milk samples in percentages of 0.29% nd 2.76% respectively.

NTRODUCTION

Besides economical losses due to brucellosis among our livestock, *B. melitensis* is a dangerous public health hazard. The organism is transmissible to man either by direct contact and management of infected animals especially sheep or through milk or milk products originated from infected animals. This results in a severe disease condition in the affected persons which is known as undulant fever. Detection of brucella-infected lactating ewes is of utmost importance for better protection of human population. ABRT and WAT have been reported as routine serological tests to identify brucella-infected animals (7,8). In more recent years, complement fixation test (CFT), and Coomb's test (WGT) were added to the list as supplemental methods, both on serum and whey samples of different animal species (1, 5, 12). However, it has been reported, by several authors, that the ABRT is not as reliable as CFT and other serological tests for laboratory diagnosis of brucellosis in sheep as in cattle (4,9,12). According to the available literature, no information was

milk for diagnosis of brucella infection. This work was carried out to evaluate the efficacy of different serological tests of milk samples, simultaneously with bacteriological examination, in the laboratory diagnosis of ovine brucellosis.

MATERIALS AND METHODS

Samples : A total of 689 milk samples was collected from ewes at different stages of lactation. The ewes represnted different regions of some northern Egyptian governorates.

Bacteriological examination: The collected milk samples were centrifuged and both sediment and cream layer were streaked onto Albimi agar plates containing ethyl violet, actidione and bacitracin. The inoculated plates were incubated at 37°C in the presence of 10% CO2 for 96 h. The suspected brucella colonies were further examined according to previously described method (16). Furthermore, the identified brucella isolates were serotyped using monospecific anti-*B. melitensis* sera obtained from Buroughs Wellcome Research Laboratory **(Beckenham, U. K.).**

Milk serological tests: ABRT was performed by mixing 1 ml of milk with 0.03 ml (one drop) of brucella haematoxylin-staiend antigen (Vet. Ser. and Vacc. Res. Inst., Abbasia, Cairo, Egypt) in a Wassermann tube. The results were recorded after 1 to 3 h incubation at room temperature (2). WAT was carried out on milk whey which was separated by addition of one drop of rennet enzyme to 5 ml of fresh milk. After centrifugation of the mixture at 900 xg for 12 min., whey was collected for WAT. Fifty percent or more agglutination of 1/1 or higher dilution of whey was considered positive for brucella infection (3). WGT (Coombs test) was also performed on whey obtained as previously mentioned. A volume of 0.8 ml of sterile physiological saline were mixed with 0.2 ml whey and incubated in a water bath at 37°C for min. The whey samples were serially diluted sterile normal saline in 0.5 ml volumes to whi equal volumes of standard *B. melitensis* strain antigen (Vet. Ser. and Vacc. Res. Inst., Abbas Cairo Egypt) were added. The mixture was inc bated at 37°C for 2 h after which 1.5 ml of ster normal saline were added to each whey-antig mixture. The antigen bacterial cells with any p sible reacting specific immunoglobulins were dimented by centrifugation and the pellet washed three times in normal saline. The wash pellet was suspended in 1 ml of rabbit anti-sh globulin at the optimum dilution* and the mixt was incubated at 37°C for 24 h. Fifty percen more aglutination of 1/10 or higher whey dilu was considered positive for brucella infec (10).

* Optimum dilution of rabbit anti-sheep globulin serum was detected by checkerboard titration against known anti-brucella positive (Federal Institute for human health protection and Veterinary Medicine, Berlin, Germany) and was calculated as the highest dilu the serum which resulted in detection of the accurate titre of the brucella positive serum.

RESULTS AND DISCUSSION

Bacteriological examination resulted in identification of 21 burcella isoaltes out of 689 tested sheep milk samples with a percentage of 3.05%. Of the 21 brucella isoaltes, 19 were typed as *B. melitensis* biotype 3, while two isolates were classified as *B. melitensis* biotype 2 (table 1). This result indicates that *B. melitensis* biotype 3 is epidemiologically and zoonotically more significant than the biotype 2. Regardless of biotyping, the incidence rate of *B. melitensis* detected in this study agreed to a large extent with rates recorded in many preceding studies (6, 14, 18) which showed incidence rates of 4.3%, 2% and 4.52% of *B. melitensis* among sheep, respectively. This shows how important is that bacteria as a public health hazard. However, if the biotyping is to be considered, *B. melitensis* biotype 3 should be respected when producing immunizing or diagnostic reagents for ovine brucellosis.

Concerning serological tests, ABRT results were compared to the results of bacteriological examination. Out of 689 ABRT-tested milk samples, 645 were negative. The rest of the samples (44) showed different positive results with the ABRT where 13.64%, 22.27% and 63.64% of the samples showed positivity degrees of 1+, 2+ and 3+, respectively. This indicates that ewes of positive or dubious results of ABRT are not necessarily positive for bacterial isoaltion. In other words, isolation of brucella organisms from ewe milk was found to be inferior to the ABRT (21/44) with more than 50% of animals might escape the bacterial isoaltion (table 2). A collective correlation of ABRT positive samples with bacterial isolation, WAT and WGT results are depicted in table 3. WAT failed to detect 8 cases, which were positive for bacterial isolation. In contrast, only 4 out of 11 whey, WAT-positive samples with 1/20 titters, were positive in cultures. Thus, the WAT can be considered not only more laborious and time consuming but also less efficient than the

Table 1: Prevalence of *B. melitensis* and its biotypes in ewe milk samples

Total* No. of milk samples	*B. melitensis* isolates **		*B. melitensis* biotypes					
	Number	Percentage	Biotype 2			Biotype 3		
			Number	Perentage/ Isolates	Percentage/ Milk samples	Number	Perentage/ Isolates	Percentage/ Milk samples
689	21	3.04%	2	952%	0.29%	19	90.48%	2.76%

* Sediment and cream layer of each sample were cultivated
** *B. melitensis* was identified using routine methods and scotyping of the isolates against monospecific *B. melitensis* antiserum

ABRT in detection of *B. melitensis* infection in lactating ewes. Similar results were previously recoreded by many investigators (3,5,12). WGT, on the other hand, showed presumably false-negative results, since 0.63% of 631 samples, which were negative for WGT, yielded *B. melitensis* in culture. However, the WGT was found to be more specific than the WAT and remained positive much more longer until a titre of 1/640. The same observation was recorded by other investigators who reported that Coomb's test was found to be more specific and remained positive much longer than the regular agglutination tests in brucella-infected sheep (12). Comparison of the three serological tests to the results of cultivation indicates

Table 2: Comparison of ABRT and bacterial isolation for diagnosis of ovine brucellosis using ewe milk samples.

Test	Total No. of milk samples	Positive samples	Percentage	ABRT intensity		
				+	++	+++
ABRT	689	44	6.38%	6	10	28
Bacteriological examination	689	21*	3.04%	2	8	11

*All samples (21) were positive with ABRT but with different intensities

Table 3: Comparison of WAT and WGT reactions in ewe milk samples of different results for *B. melitensis* isolation.

			No. of milk samples	Serological reaction of 21-*B. melitensis*-positive milk-samples
*Results of WAT	Negative samples		456	8
		1/10	11	4
		1/20	8	3
	Positive samples (dilution)	1/40	7	2
		1/80	6	2
		1/160	2	1
		1/320	1	1
** Results of WGT	Negative samples		631	4
		1/10	16	3
		1/20	18	2
		1/40	14	5
	Positive samples (dilution)	1/80	5	3
		1/160	3	2
		1/320	1	1
		1/640	1	1

* WAT means whey agglutination test.
** WGT means whey antiblobulin test.

that ABRT might be considered as the most reliable test for diagnosis of ovine brucellosis. This reliability can be mostly referred to the fact that the ABRT is easier to perform and less time consuming than the other tests (1,9). Additionally, ABRT seems to be the recommended test if public health is considered, as the test was able to detect culture-negative brucella-shedding ewes. Our findings agree to a large extent with the findings of some previous studies. In one survey, it was found that out of 173 ABRT-positive sheep milk samples only 68 yielded *B. melitensis* on culturing (4) . Some milk samples were positive for all the serological tests while negative for *B. melitensis* isolation. This is not surprising because the same observation was previously recorded by many investigators who encountered low incidence of brucella infection (0.0-2.0%) when their results relied only on bacterial isolation (1,6,13,17). The serological tests, therefore, remain the most reliable diagnostic methods for ovine brucellosis.

In conclusion, serological investigation can be carried out on sheep milk samples so as to take rapid action in order to minimize spread of brucellosis to other animals and protect human poulation from undulant fever due to consumption of contaminated milk products of sheep or managing infected animals. However, no single serological test can be trusted to detect every infected sheep, but we agree with other workers who declared out the importance of at least two positive serological tests to indicate incidence of brucellosis in sheep (11,13,15,16).

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