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DIAGNOSIS OF BRUCELLA INFECTION IN DAIRY CATTLE WITH SEROLOGICAL TESTS IN ASSIUT GOVERNORATE

(With 7 Tables and 2 Figures)

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تشخيص عدوى البروسيلا باستخدام اختبارات سيرولوجية في الحيوانات الحلابة بمحافظة أسيوط

توفيق البسيونى ، إيناس البرنس ، سهير زين العابدين ، أنسى صادق

يعتبر مرض البروسيلا من الأمراض المعدية التي لها أهمية كبري خاصة من الناحيتين الصحية والاقتصادية، فهو يسبب ما يعرف بحمي مالطة في الإنسان بالإضافة إلي الإجهاض المتكرر في الحيوانات مما يؤدي إلي خسائر اقتصادية ضخمة نتيجة فقد الأجنة ونقص محصول اللبن بالإضافة إلي قلة الخصوبة في الذكور، هذا وتعتبر وسائل التشخيص السيرولوجية من أهم الوسائل لاكتشاف المرض بين مختلف أنواع الحيوانات. ونظراً لما لهذا الميكروب من تأثير مباشر علي صحة الإنسان فقد تم جمع عدد ٢١٠ عينة من ألبان الأبقار و٥٠ عينة من ألبان الجاموس من أماكن مختلفة بمحافظة أسيوط بغرض إجراء اختبار اللبن الحلقي واللبن الحلقي بالتخفيف علي اللبن الخام وإجراء اختبارات الروزبنجال واختبار القاعدي المحمض الشريحي المتوازن واختبار الريفانول واختبار التلازن الأنبوبي البطيء علي شرش هذه العينات. وبفحص عينات لبن وشرش لبن الأبقار كانت نتائج اختبار اللبن الحلقي إيجابية بنسبة ١٢.٣٨% ، اشتباه بنسبة ٧.٦٢% وسلبية بنسبة ٨٠%. وكانت نتائج الاختبارات السيرولوجية بالنسبة لشرش اللبن المختبرة بواسطة اختبارات الروزبنجال ، القاعدى المحمض الشريحي المتوازن، الريفانول والتلازن الأنبوبي البطيء ايجابية بنسب ٤.٢٩ ، ٤.٢٩ ، ٤.٢٩ و ٥.٢٤% على التوالي. أما بالنسبة لعينات لبن وشرش اللبن في الجاموس فكانت جميع اختبارات اللبن الحلقي واختبارات الشرش سلبية. هذا وقد تمت مناقشة الأهمية الصحية والوبائية والاقتصادية لميكروبات البروسيلا في الإنسان والحيوان والشروط الواجب إتباعها في منع انتشاره واختباره في مزارع الألبان المختلفة لدرء خطرها على الإنسان.

SUMMARY

A total of 260 of raw milk samples were collected from different localities in Assiut Governorate. These samples represented by 210 and

50 each of raw milk as well as milk whey samples obtained from cows and buffaloes, respectively. The incidence of brucella antibodies in milk samples were estimated by MRT and by wRBPT, wBAPAT, wRiv.T and wTAT in their corresponding whey samples. Out of 210 cows milk samples examined by MRT, 12.38% were positive (constituting 4.76, 2.38 and 5.24% were positive in grade (++), (+++) and (++++), respectively), 7.62% were doubtful and 80% were negative. In the corresponding milk whey samples by whey serological tests: wRBPT, wBAPAT, wRiv.T and wTAT gave 4.29, 4.29, 4.29 and 5.24% positive, while, the negative results were 95.71, 95.71, 95.71 and 94.76%, respectively. In case of buffalo's milk, all of the examined milk as well as milk whey samples found to be negative to MRT as well as to all whey serological tests.

Key words: *Diagnosis, brucella infection, dairy cattle serological tests, Assiut Governorate.*

INTRODUCTION

Brucellosis is still one of the most important zoonotic diseases of both public health and economic importance in most developing countries and recognized as a major milk borne disease in human beings. It is caused by one of the following four species of Gram-negative, facultative, intracellular coccobacilli: *Brucella melitensis*, *Brucella abortus*, *Brucella suis*, or *Brucella canis* (Radolf, 1994 and Wallach *et al.*, 1997*).* Unfortunately, infected animals such as sheep, goats, cows, buffaloes and camels excrete brucella organisms in their milk sporadically throughout the entire period of lactation, in counts varied from a few to up 15000 cells/ml milk as previously stated by many investigators (Awad *et al.*, 1975 and El-Gibaly *et al.*, 1981). Moreover, it is a source of serious economic losses of animal industry due to abortion, losses of calves, reduction in milk yield by 7-20%, some breeding troubles in infected animals and veterinary costs of diagnosis and control measures (Shalaby, 1986; Adawy, 1989; Sanders, 1989 and Soliman, 1998). Furthermore, brucella organisms can be transmitted from infected animals to man by ingestion of unpasteurized milk and milk products, by contact with infected animals or their discharges, or by inhalation of aerosols containing brucella organisms (El-Amin *et al.*, 2001). Therefore, unpasteurized milk, cream, butter, unfermented cheese and other products made from untreated milk constitute a serious health hazard in area where brucella infection is widespread in dairy animals.

The presence of brucella organisms in milk have conducted by several investigators (Hamdy, 1989; Hamdy, 1992; Soliman, 1998; Abdel-Hakiem, 1999; Abd-Alla *et al.*, 2000; and Abdel-All, 2001).

The definitive diagnosis for brucellosis requires the recovery of the organism, however; it is difficult to recover from life infected animals, therefore, diagnosis has been based mostly on the results of serological tests (Hamdy, 1997). It is easier for using milk and milk whey for diagnosing brucellosis as injuring animals for collecting blood samples is difficult (Farag, 1998).

The milk ring test (MRT) for diagnosing brucellosis depends on the presence of brucella agglutinins in milk which may be present in milk before blood. Also, it could detect developing infection earlier than blood serum agglutination test (Lerche, 1949 and Molem *et al.*, 1950). In addition, milk ring test (MRT) alone was sufficient to detect all cases of brucellosis and the additional periodic blood tests were unnecessary due to high sensitivity of the test in detection of infected animals and its usefulness as a screening test (Nicoletti & Bruch, 1969).

In the recent years, attention has been directed to restudy the prevalence of brucellosis in Egypt due to continuous importation of large numbers of cattle and establishment of several dairy farms with extensive cattle population among animals (Adawy, 1985). Therefore, the aim of this work was performed to determine of the incidence of brucella organisms in raw milk as well as milk whey of cows and buffaloes by using different serological tests.

MATERIALS and METHODS

1- Milk samples:

A total of 260 random milk samples were obtained from different localities in Assuit Governorate, comprising 210 samples from lactating cows and 50 samples from lactating buffaloes.

2- Whey Milk samples:

Milk whey was prepared from the collected milk samples according to Morgan *et al.* (1978).

3- Antigens:

All the antigens used throughout the work were obtained from Veterinary Sera and Vaccines Research Institute, Abassia, Cairo, Egypt. These antigens included:-

a - Milk ring test antigen (Haematoxyline blue stained).

b - Rose Bengal plate test antigen.

c - Buffered acidified plate test antigen.
d - Rivanol test antigen.
e - Tube agglutination test antigen.

Milk ring test (MRT) for cow's and buffalo's milk and whey buffered acidified plate antigen test (wBAPAT) were carried out according to Alton *et al.* (1988). Serial dilution milk ring test and whey Rivanol test were performed according to National Veterinary Services Laboratories, Ames, Iowa, USA (1984). While, whey Rose Bengal plate test was carried out according to Morgan *et al.* (1978) and Alton *et al.* (1988) and whey tube agglutination test (wTAT) was estimated by European method described by Morgan (1967).

RESULTS

The obtained results are recorded in Tables 1-7 and Fig. 1 &2.

Table 1: Incidence of brucella antibodies in cow's milk samples based on results of milk ring test (MRT)

No. of samples	Positive reactors								Doubtful +ve		Negative	
	Positive rating						Total		No.	%	No.	%
	++		+++		++++		No.	%				
	No.	%	No.	%	No.	%						
210	10	4.76	5	2.38	11	5.24	26	12.38	16	7.62	168	80

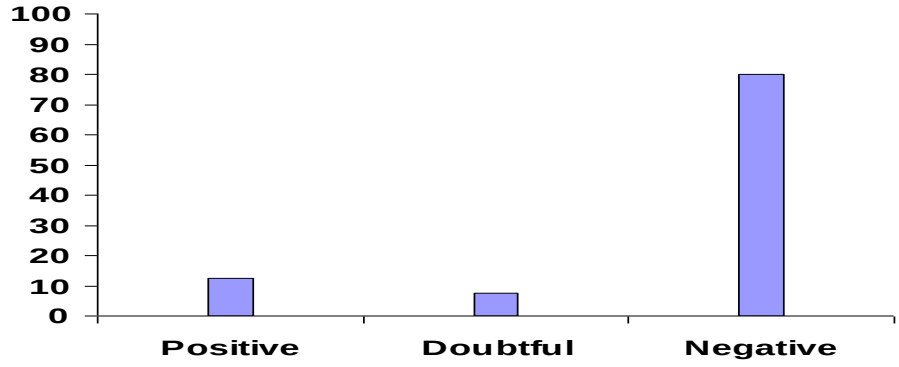

Fig. (1): Incidence of brucella antibodies in cattle milk samples based on results of milk ring test(MRT).

Table 2: Statistical analysis of serial dilution milk ring test (MRT) titres and its relationship with grade of MRT.

Titres	1:1		1:2		1:4		1:8		1:16		1:32		1:64		1:120		1:256		1:512		1:1024	
No. of samples	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	10	4.76	2	0.95	0	0	3	1.43	1	0.48	3	1.43	2	0.95	1	0.48	0	0	2	0.95	2	0.95
Grade of MRT	++		+++				++++		++++		++++		++++		++++				++++		++++	

++ = Positive +++ = Strong positive ++++ = Very strong positive

Table 3: Incidence of brucella antibodies in cow's milk whey samples based on results of whey serological tests.

No. of samples	wRBPT				wBAPAT				wRiv. T				wTAT			
	Positive		Negative		Positive		Negative		Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
210	9	4.29	201	95.71	9	4.29	201	95.71	9	4.29	201	95.71	11	5.24	199	94.76

wRBPT = whey Rose Bengal Plate Test wBAPAT = whey Buffered Acidified Plate Antigen Test
wRiv. T = whey Rivanol Test wTAT = whey Tube Agglutination Test

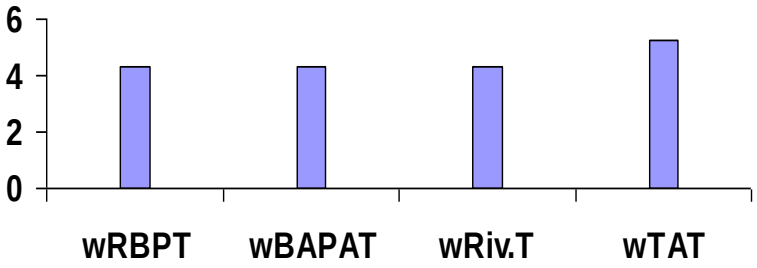

Fig. (2): Incidence of brucella antibodies in cattle milk whey samples based on results whey serological tests.

Table 4: Different titres of whey Rivanol test (wRiv.T) on cow's milk whey samples.

No. of examined samples	Titres of whey Rivanol test										Total			
	1/25		1/50		1/100		1/200		1/400		Reactors		Non-reactors	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
210	1	0.48	0	0	2	0.95	2	0.95	4	1.91	9	4.29	201	95.71

Table 5: Different titres of whey tube agglutination test (wTAT) on cow's milk whey samples.

No. of examined samples	Titres of whey tube agglutination test																Total			
	1/10		1/20		1/40		1/80		1/160		1/320		1/640		1/1280		Reactors		Non-reactors	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
210	4	1.9	2	0.95	0	0	0	0	1	0.48	1	0.48	2	0.95	1	0.48	11	5.24	199	94.76

Table 6: Incidence of brucella antibodies in buffalo's milk samples based on results of milk ring test (MRT).

No. of examined samples	Positive		Negative	
	No.	%	No.	%
50	0	0	50	100

Table 7: Incidence of brucella antibodies in buffalo's milk whey samples based on results of whey serological tests.

No. Of samples	wRBPT				wBAPAT				wRiv. T				wTAT			
	Positive		Negative		Positive		Negative		Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
50	0	0	50	100	0	0	50	100	0	0	50	100	0	0	50	100

wRBPT = whey Rose Bengal Plate Test wBAPAT = whey Buffered Acidified Plate Antigen Test
wRiv.T = whey Rivanol Test wTAT = whey Tube Agglutination Test

DISCUSSION

Cow's milk samples:

Data presented in Table 1 and Fig. 1 show the incidence of brucella antibodies in the examined cow's milk samples based on the results of milk ring test (MRT). Out of 210 samples, 26 (12.38%) were positive constituting 10 (4.76%) of grade (++), 5 (2.38%) of grade +++ (strong positive) and 11 (5.24%) of grade ++++ (very strong positive). While, 16 (7.62%) were doubtful and the remaining 168 (80%) were negative. Nearly similar results were recorded by Salem *et al.* (1987) who detected brucellosis in a prevalence of 11.4% in cows by milk ring test (MRT). Lower results were stated by Awad *et al.* (1977), Robertson *et al.* (1980), Shaw (1986), Bastawrous (1987), Hosein & El-Kholy (1993), Gandara *et al.* (1994), Kadry (1996), Rasch *et al.* (1997), Abdel-Hakiem (1999), Abd-Alla *et al.* (2000) and Türütoğlu *et al.* (2003). They detected incidences of 0.2, 8.42, 1.71, 4, 4.1, 4.31, 0.99, 4.50, 8, 3.67 and 3%, respectively. While, higher results were estimated by Hamdy (1989), El-Sheary (1993), Youssif (1994), Hamdy (1997) and Abdel-All (2001) who stated incidences of 68.13, 29.2, 33.6, 66.6 and 38.33%, respectively.

The relatively higher results obtained in this study could be attributed to the fact that MRT is highly sensitive, rapid screening test (Nicoletti & Bruch, 1969 and Salem *et al.*, 1987) and could detect developing infection earlier than the blood serum agglutination test (Molem *et al.*, 1950). Furthermore, instead of the MRT which depends on the presence of brucella agglutinins in milk (Lerche, 1949), some false positive results may be obtained due to treatment of animals with Estrumate, regressing corpus luteum, milk from late lactation period or

in case of subclinical mastitis (Morgan *et al.,* 1978 and Alton *et al.,* 1988).

Results illustrated in Table 2 showed that, out of the 210 examined samples, 10 (4.76%) gave a titre of 1:1 and the grade of MRT of this sample was ++, while, 2 (0.95%) and 3 (1.43%) gave titres of 1:2 and 1:8, respectively and their MRT grade was +++ (strong positive). Moreover, 1 (0.48%), 3 (1.43%), 2 (0.95%), 1 (0.48%), 2 (0.95%) and 2 (0.95%) of samples gave titres of 1:16, 1:32, 1:64, 1:128, 1:512 and 1:1024, respectively, and their grade of MRT was ++++ (very strong positive). Samples which having titres of serial dilution milk ring test 1:16 or above may referred to the presence of brucella organisms in milk (Alton *et al.,* 1975).

In addition, it is clear that, the examined samples which gave a titre of 1:1, were only of grade ++ of MRT. This could be attributed either to that these samples came from animals having low blood serum titres (1:40 or less) and by dilution with normal milk, the reaction disappeared due to dilution of agglutinins (Awad *et al.,* 1977) or to the presence of some false positive (MRT) results and by addition of normal milk the reaction disappeared. Also, from Tables 1 & 2, it is evident that samples of grade +++ (strong positive), showed titres ranged from 1:2 to 1:8, however, at titres of 1:16 up to 1:1024 the samples were of grade ++++ (very strong reaction of MRT). These findings indicated that, some positive samples to MRT still gave reaction up to 1:1024 dilution with normal milk, which coincident with that stated by (Awad *et al.,* 1977; El-Gibaly *et al.,* 1991; El-Sheary, 1993; Youssif, 1994 and Abdel-Hakiem, 1999). It is worthy to mention that, the very high titres (1:1024) indicated that the infection is localized in the udder (Meador *et al.,* 1989) or these animals having high titres of blood serum agglutinins, as the milk titres agglutinins correspondingly increase with those of blood serum (El-Gibaly *et al.,* 1991).

The incidence of brucella antibodies in the examined cow's milk whey samples based on the results of whey serological tests was recorded in Table 3 and Fig. 2. Out of 210 samples examined by whey Rose Bengal plate test (wRBPT), 9 (4.29%) were positive while, 201 (95.71%) were negative. The positive result was in accordance with that obtained by Abdel-Hakiem (1999) who recorded 4.7% of positive reactors. While, somewhat lower result was stated by Abd-Alla *et al.,* 2000 (3.53% positive reactors and 96.46% negative). However, relatively higher results were estimated by Abdel-Rahman, 1991 (22.1%) and Hamdy, 1997 (39.2%).

Concerning whey buffered acidified plate antigen test (wBAPAT), the results in Table 3 and Fig. 2 showed that, 9 (4.29%) and 201 (95.71%) of examined samples gave positive and negative reactors, respectively. Higher results were recorded by Abdel-Rahman, 1991 (38.9%) and Hamdy, 1997 (54.9%). With regard to whey Rivanol test (wRiv.T), 9 (4.29%) and 201 (95.71%) of examined samples gave positive and negative reactors, respectively. Nearly a similar finding was recorded by Abdel-Hakiem (1999) who pointed out that wRiv.T gave 4% positive reactors. However, a lower result was estimated by Abd-Alla *et al.,* 2000 (2.86% positive reactors and 97.14% negative). Moreover, the whey tube agglutination test (wTAT) gave 11 (5.24%) samples as positive reactors and 199 (94.76%) as non-reactors. Comparatively, lower result was estimated by Türütoğlu *et al.,* 2003 (2.2% positive reactors). In the contrary, a relatively higher percentage (49%) was reported by Hamdy (1997).

By comparing the results which obtained by wTAT and those for wRBPT, wBAPAT and wRiv.T, it is evident that wTAT is the relatively most sensitive one as it gave 5.24% positive reactors, while, it was 4.29% for each wRBPT, wBAPAT and wRiv.T. These findings could be attributed to that, the wTAT detect all immunoglobulins (IgG, IgA and IgM) (FAO/WHO, 1986 and Saleh *et al.,* 2003), this render wTAT is the relatively most sensitive whey serological test. Also, the whey samples which gave negative wRBPT and wRiv.T and positive wTAT, may be in the early stage of infection where the IgM is the most predominante isotype in this stage (Morgan, 1967). This immunoglobulin not detected by Riv.T and RBPT due to precipitating effect of Rivanol solution (2-ethoxy-6,9-diaminoacridine lactate) on IgM in the former and due to the inhibitory effect of acidic pH (3.65) on IgM in the later (Morgan, 1967; Davies, 1971 and Pietz & Cowart, 1980). The low pH of BAPAT (4.0) may have some inhibitory effect on IgM and this render the test is less sensitive than wTAT.

Table 4 showed different titres of whey Rivanol test on the examined whey samples where, 1 (0.48%), 2 (0.95%), 2 (0.95%) and 4 (1.91%) of samples gave positive results in titres of 1/25, 1/100, 1/200 and 1/400, respectively. The higher titres (1/400) indicated that, these samples may came from chronically infected animals (late stage of infection) as the Rivanol test determines only the agglutinating activity of the IgG isotype which produced later in infection (FAO/WHO, 1986 and Alton *et al.,* 1988). By performing wTAT, 4 (1.9%), 2 (0.95%), 1 (0.48%), 1 (0.48%), 2 (0.95%) and 1 (0.48%) of samples showed titres

of 1/10, 1/20, 1/160, 1/320, 1/640 and 1/1280, respectively, as recorded in Table 5. The higher titres (1/640 and 1/1280), indicated that these samples came from animals in which the infection is localized in the udder (Meador *et al.,* 1989) or these animals having high titres of blood serum agglutinins as the milk agglutinins correspondingly increase with those of blood serum (El-Gibaly *et al.*, 1991).

It is worthy to state that, the low sensitivity of wRBPT, wBAPAT, wRiv.T and wTAT in comparison to MRT (Tables 1 and 3), could be attributed to certain factors such as removal of solid part in milk with rennin, the changes in pH, changes in the molecular weight of some immunoglobulins and the majority presence of immunoglobulins in the cream layer of raw milk. Therefore, the whey contains less amount of immunoglobulins in comparison to raw milk with cream (Hamdy, 1997; Abdel-Hakiem, 1999 and Abd-Alla *et al.*, 2000). In addition, the whey tests are less sensitive, but less influenced by non-specific factors than MRT and give more confirmatory results (Morgan *et al.,* 1978; El-Gibaly *et al.,* 1990 and Hamdy, 1997).

Buffalo's milk samples:

From Table 6, it is evident that, all examined buffalo's milk samples were negative to MRT. This result goes parallel with that reported by Awad *et al.* (1977) who estimated 3003 lactating buffaloes by MRT and the results were negative. Also, wRBPT, wBAPAT, wRiv.T and wTAT showed negative results to all examined buffalo's milk whey samples (Table 7). The negative results of MRT and whey serological tests in milk as well as milk whey samples may be attributed to that, these samples may came from animals with low blood serum agglutination titres (1/40 or less) (Awad *et al.,* 1977) or the buffaloes are somewhat resistant to brucellosis. In addition, one can recommend that, MRT and whey serological tests in buffaloes milk as well as milk whey need further investigation.

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