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SEROLOGICAL STUDIES ON SHEEP AND GOATS' MILK FOR DIAGNOSIS OF BRUCELLA INFECTION IN ASSIUT GOVERNORATE

(With 5 Tables and 2 Figures)

By

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دراسات سيرولوجية على لبن الأغنام والماعز لتشخيص عدوى البروسيلا بمحافظة أسيوط

توفيق البسيونى , إيناس البرنس , سهير زين العابدين , أنسى أديب صادق

ارتبطت الألبان ومنتجاتها بحياة الإنسان والحيوان لما لها من قيمه غذائية عالية وهبها الله إياها، إلا أنها قد تتسبب في نقل بعض الأمراض المعدية ومنها مرض البروسيلا الذي له 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 في كمية إدرار اللبن بالإضافة إلي قلة الخصوبة في الذكور, هذا وتعتبر وسائل التشخيص السيرولوجية من أهم الوسائل لاكتشاف المرض بين أنواع الحيوانات المختلفة. ونظراً لما لهذا الميكروب من تأثير مباشر علي صحة الإنسان فقد استهدفت هذه الدراسة إجراء اختبارات سيرولوجية للكشف عنه، وذلك بجمع عدد ١٢٠ عينة من كل من ألبان الأغنام والماعز من قرى 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. وقد كانت نتائج اختبار اللبن الحلقي في عينات لبن وشرش اللبن في الأغنام ايجابية بنسبة ١٠%. وأعطت الاختبارات السيرولوجية لشرش اللبن نتائج ايجابية في اختبارات الروزبنجال، القاعدي المحمض الشريحى المتوازن، الريفانول والتلازن الأنبوبي البطيء بنسب ١,٦٧ ، ١,٦٧ ، ٣,٣٣ و ١,٦٧% على الترتيب. أما بالنسبة لعينات لبن وشرش اللبن في الماعز فقد كانت نتائج اختبار اللبن الحلقي ايجابية بنسبة ١٥%. وكانت نتائج الاختبارات السيرولوجية بالنسبة لشرش اللبن في اختبارات الروزبنجال، القاعدي المحمض الشريحى المتوازن، الريفانول والتلازن الأنبوبي البطيء ٣,٣٣ ، ٣,٣٣ ، ٢,٥ و١,٦٧% على الترتيب .هذا وقد تمت مناقشة الأهمية الصحية والوبائية والاقتصادية لميكروبات البروسيلا في الحيوان والإنسان والشروط الواجب إتباعها لمنع انتشارها واختبارها في مزارع الألبان المختلفة لدرء خطرها.

SUMMARY

Two hundred and forty random raw milk samples were collected from sheep and goats at different villages in Assiut Governorate. These samples represented by 120 each of raw milk as well as milk whey samples for each sheep and goat. The incidence of brucella antibodies in milk samples were estimated by milk ring test (MRT) and by whey Rose Bengal plate test (wRBPT), whey buffered acidified plate antigen test (wBAPAT), whey Rivanol test (wRiv.T) and whey tube agglutination test (wTAT) in their corresponding whey samples. In case of sheep milk samples examined by MRT, 2.5 and 7.5% gave positive ring and ring & disc, respectively, with 10% total positive and 90% negative. While, in milk whey samples, wRBPT, wBAPAT, wRiv.T and wTAT gave 1.67, 1.67, 3.33 and 1.67% positive results. Concerning goat's milk samples, it is evident that 2.5, 10.83 and 1.67% were positive by MRT showing ring, ring & disc and disc, respectively, with total positive results of 15%. Moreover, whey serological tests wRBPT, wBAPAT, wRiv.T and wTAT gave 3.33, 3.33, 2.5 and 1.67% positive results, respectively.

Key words: *Serology, brucella, sheep, goats, milk.*

INTRODUCTION

Animal and human health are inextricably linked as people depend on animals for nutrition, socio-economic development and companionship. Yet animals can transmit many diseases to humans which are potentially devastating. Brucellosis is a zoonotic disease of both public health and economic significance in most developing countries and recognized as a major milk borne disease in human beings (WHO, 2007). Six species of brucella are currently known, of which *Brucella melitensis*, *Brucella suis* and *Brucella abortus*, have public health implications (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 (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 off-springs, reduction in milk yield by 7-20%, some breeding troubles in infected animals and veterinary costs of diagnosis and control measures (Shalaby, 1986; 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 unheat-treated 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; Abdel-All, 2001 and Hamdy & Amin, 2002).

The definitive diagnosis for brucellosis requires the recovery of the organisms, 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 are difficult (Farag, 1998).

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, 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).

Worldwide, brucellosis remains a serious zoonotic disease where *Brucella melitensis* is endemic in sheep and goats (Massis *et al.*, 2005), so attention has been directed to restudy its prevalence in Egypt. Therefore, the aim of this work was performed to determine the incidence of brucella organisms in raw milk as well as milk whey of sheep and goats by using different serological tests.

MATERIALS and METHODS

1- Milk samples:

Two hundred and forty random raw milk samples were collected from sheep and goats at different villages in Assiut Governorate. These samples represented by 120 each of raw milk as well as milk whey samples for each lactating sheep and goat.

2- Whey Milk samples:

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

3-Antigens:

All antigens used 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.

MRT for sheep and goat's milk and wBAPAT were carried out according to Alton *et al.* (1988). Serial dilution MRT and wRiv.T were performed according to National Veterinary Services Laboratories, Ames, Iowa, USA (1984). While, wRBPT was carried out according to Morgan *et al.* (1978) and Alton *et al.* (1988) and wTAT was estimated by European method described by Morgan (1967).

RESULTS

The obtained results are recorded in Tables 1-5 and Figures 1& 2.

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

Types of animals	No. of examined samples	Positive								Negative	
		Ring		Disc		Ring +Disc		Total			
		No.	%	No.	%	No.	%	No.	%	No.	%
Sheep	120	3	2.5	-	-	9	7.5	12	10	108	90
Goat	120	3	2.5	13	10.83	2	1.67	18	15	102	85

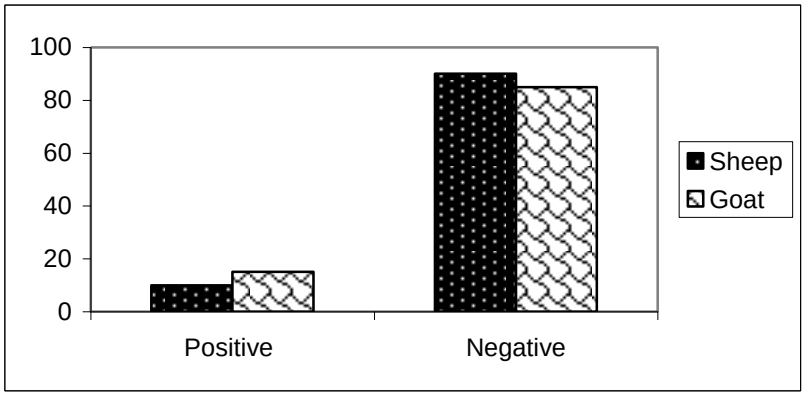

Fig. 1: Incidence of brucella antibodies in sheep and goat's milk samples based on results of milk ring test (MRT).

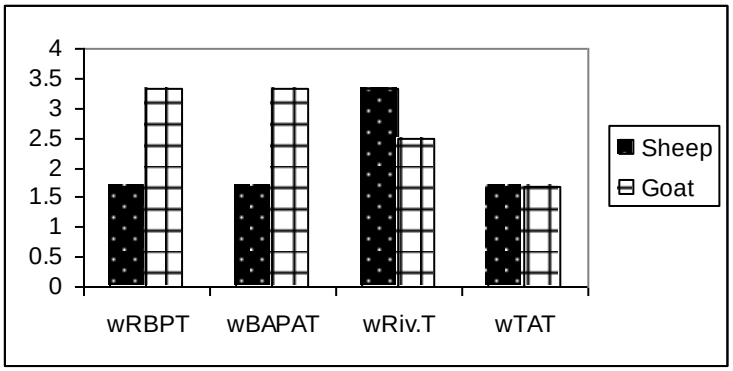

Fig. 2: Incidence of brucella antibodies in sheep and goat's milk whey samples based on results of whey serological tests.

Table 4: Different titers of whey Rivanol test (wRiv.T) on sheep and goat's milk whey samples.

Types of animals	No. of examined samples	Titers 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.	%
Sheep	120	1	0.83	1	0.83	1	0.83	1	0.83	-	-	4	3.33	116	96.67
Goat	120	-	-	1	0.83	-	-	1	0.83	1	0.83	3	2.5	117	97.5

Table 5: Different titers of whey tube agglutination test (wTAT) on sheep and goat's milk whey samples.

Types of animals	No. of examined samples	Titers of whey tube agglutination test								Total			
		1/10		1/20		1/40		1/80		Reactors		Non – reactors	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Sheep	120	1	0.83	-	-	1	0.83			2	1.67	118	98.33
Goat	120	-	-	-	-	1	0.83	1	0.83	2	1.67	118	98.33

DISCUSSION

Sheep milk samples:

Table 1 and Fig. 1 showed the incidence of brucella antibodies in the examined sheep milk samples based on the results of MRT. Out of 120 milk samples, 12 (10%) gave positive reaction to MRT. Similar results were reported by Abdel-All (2001) who recorded that, the incidence of brucella antibodies in the examined sheep milk samples based on the results of MRT was 10%. While, lower findings were obtained by Awad *et al.* (1975); Bubey & Mathur (1980); Abd El-Ghani

et al. (1983); Bastawrous (1987) and Masoumi *et al.* (1992) who estimated incidences of 5, 2.38, 1.73, 2.06, 2.4 and 7.6% in sheep milk, respectively. In the contrary, higher results were recorded by Youssif (1994) and Türütoğlu *et al.* (2003) who stated that, by applying MRT on sheep milk samples, 33.3 and 17.7% were positive, respectively. Also, from Table 1 the behavior of MRT indicated that, 3 (2.5%) of samples showed ring formation, while, 9 (7.5%) showed both ring and disc formation. These results may be attributed to the size of fat globules, as in milk samples with large fat globules (av. 6.6±0.9 mum diam.) a ring was observed, but in samples with medium fat globules (av. 4.4±1.1 mum diam.) a disc and ring occurred together, while in samples with small fat globules (av. 2.8±0.6 mum diam.) only a disc was seen (Soni, 1979). Due to the little information and literature about the behavior of MRT in sheep milk samples, the test needs further investigation.

From the results of serial dilution MRT using normal milk (Table 2), it is clear that, 1 (0.83%) , 3 (2.5%), 3 (2.5%), 1 (0.83%), 2 (1.67%), 1 (0.83%) and 1 (0.83%) of examined samples gave titers of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32 and 1:128, respectively, with total reactors of 12 (10%) while, the remaining 108 (90%) samples were non-reactors. Samples which having titers of serial dilution milk ring test 1:16 or above may referred to the presence of brucella organisms in milk (Alton *et al.,* 1975). Also, the higher titers in some samples indicated that, positive samples still gave MRT reaction even when diluted with brucella antibodies free milk up to 1:128.

Results illustrated in Table 3 and Fig. 2 showed that out of 120 samples examined by wRBPT, wBAPAT and wTAT, 2 samples (1.67%) of each were positive and 118 (98.33%) were negative, while wRiv.T gave 4 (3.33%) positive reactors and 116 (96.67%) non-reactors. Türütoğlu *et al.* (2003) recorded higher prevalence of 13.7% in sheep milk whey by wTAT. Furthermore, wRiv.T was the most sensitive test for detecting brucella antibodies in sheep milk whey samples as it gave 3.33% positive, while, the other tests (wRBPT, wBAPAT and wTAT) gave 1.67% positive for each. These samples which gave positive Riv.T, while negative to other tests may be obtained from chronic stage of infection, where incomplete or blocking IgA class immunoglobulin are formed (Colak *et al.,* 1992). The incomplete or blocking immunoglobulin reacted with the antigen but the reaction is not completed to cause visible clumping (Morgan, 1967). But treatment of whey samples by Rivanol solution before performing the test, precipitate

the incomplete or blocking IgA class immunoglobulin. Therefore, there is a chance for the reaction to appear.

From the data outlined in Tables 1 and 3, it is worthy to state that the low sensitivity of wRBPT, wBAPAT, wRiv.T and wTAT in comparison to MRT could be attributed to certain factors such as removal of solid part in milk with rennin, the change in pH, changes in the molecular weight of some immunoglobulins and the majority presence of immunoglobulin in the cream layer of raw milk. Therefore, the whey contains less amount of immunoglobulin in comparison to raw milk with cream (Sutra *et al.,* 1986; 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 milk ring test and give more confirmatory results (Morgan *et al.,* 1978; El-Gibaly *et al.,* 1990 and Hamdy, 1997).

By applying wRiv.T on 120 sheep milk whey samples, 1 (0.83%) gave titer of each 1/25, 1/50, 1/100 and 1/200 as obtained in Table 4. The higher titer (1/200) that was given by 1 (0.83%) sample indicated that this sample came from late stage of chronically infected animal as the Riv.T determines only the agglutinating activity of the IgG isotype which produced later in infection (FAO/WHO, 1986 and Alton *et al.*, 1988). In case of wTAT, 1 (0.83%) sample gave titer of each 1/10 and of 1/40. Moreover, 2 (1.67%) and 118 (98.33%) of tested samples were reactors and non-reactors, respectively (Table 5).

Goat's milk samples:

The incidence of brucella antibodies in the examined goat's milk samples based on the results of MRT were recorded in Table 1 and Fig. 1. Out of 120 samples, 18 (15%) were positive constituting 3 (2.5%) showed ring, 13 (10.83%) showed disc and 2 (1.67%) showed both ring and disc formation. Lower findings were recorded by Awad *et al.* (1975); Nada (1979); Bubey & Mathur (1980); Abd El-Ghani *et al.* (1983); Bastawrous (1987); Masoumi *et al.* (1992) and Mohammad (2001). They stated percentages of 3.66, 3.05, 7.25, 3.96, 3.49, 9.10 and 0.68%, respectively. In contrast, relatively higher results were estimated by Ibrahim (1990) and Abdel-All (2001) who recorded incidences of 28.6 and 21.66%, respectively.

The behavior of MRT may be attributed to the size of fat globules as well as milk immunoglobulin may have a role as IgA and IgM induce the formation of a broad colored ring after one hour incubation at 37^{o}C. While under the same conditions, no ring was observed with IgG1 or IgG2 but the colored agglutinated bacteria were

visible at the bottom of the tube. Whereas, when the four immunoglobulins were simultaneously present in milk, the formation of colored ring prevented the formation of a deposit of agglutinated bacteria on the bottom of the tubes (Collin, 1976). Owing to the few literature about the behavior of MRT in goat's milk samples, more studies must be carried out.

It is evident that, 3 (2.5%), 11 (9.17%), 1 (0.83%), 2 (1.67%) and 1 (0.83%) of goat's milk samples gave titers of 1:1, 1:2, 1:4, 1:16 and 1:64, respectively, with total reactors of 18 (15%), while the remaining 102 (85%) were non-reactors (Table 2). The higher serial MRT titers samples indicated that these samples came either from animals in which the infection is localized in the udder (Meador *et al.*, 1989) or from animals having a high blood serum titers of agglutinin as the agglutination titers of milk samples increased when corresponding blood serum titers increased as postulated by El-Gibaly *et al.* (1991).

Out of 120 samples examined by wRBPT and wBAPAT, it is noted that, 4 (3.33%) were positive while, 116 (96.67%) were negative of each as recorded in Table 3 and Fig. 2. Lower results were estimated in goat's blood serum by Bekele & Kasali (1990); Abdel-Kader (1996); Bassiony & Ibrahim (1997) and Mohammad (2001). However, higher findings were recorded in goat's blood serum by Awad *et al.* (1975); El-Bayuomy (1989) and Shalaby *et al.* (2003).

With regard to wRiv.T, 3 (2.5%) were positive and 117 (97.5%) were negative. Lower results were estimated in goat's blood serum by Abdel-Kader (1996) and Mohammad (2001), while, higher incidence was recorded by El-Bayuomy (1989).

In case of wTAT, 2 samples (1.67%) were positive while, 118 (98.33%) were negative. Lower results were obtained in goat's blood serum by Abdel-Kader (1996) and Mohammad (2001). However, a higher incidence of 4.10% was estimated by Nada (1979) in goat's milk whey.

Moreover, from Table 3 and Fig. 2, it is clear that, wTAT was the least sensitive test for the examined goat's milk whey samples as it gave 1.67% positive results, while, wRBPT, wBAPAT and wRiv.T gave positive reactions in percentages of 3.33, 3.33 and 2.5%, respectively. These findings coincided with that obtained by Fensterbank (1986) who concluded that the TAT sensitivity is low in small ruminants. In addition, the test may be negative in the early stage of infection and in old long-standing chronic infections (Morgan, 1967).

By applying wRiv.T on 120 goat's milk whey, one sample (0.83%) showed titer of each 1/50, 1/200 and 1/400 as outlined in Table 4. Worthwhile, the higher titer of 1/400 that showed in one sample indicated that this sample came from late stage of chronically infected animal as Riv.T determines only the agglutinating activity of the IgG isotype which produced later in infection (FAO/WHO, 1986 and Alton *et al.*, 1988).

The different titers of wTAT on the examined goat's milk whey samples were summarized in Table 5 where, one sample (0.83%) of titer 1/40 and one sample (0.83%) of titer 1/80 with total reactors of 2 (1.67%), while the remaining 118 (98.33%) were non-reactors.

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Table 2: Results of serial dilution milk ring test in sheep and goat's milk samples using normal milk.

Types of animals	No. of examined samples	Titers of serial dilution milk ring test																Total			
		1/1		1/2		1/4		1/8		1/16		1/32		1/64		1/128		Reactors		Non-reactors	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Sheep	120	1	0.83	3	2.5	3	2.5	1	0.83	2	1.67	1	0.83	-	-	1	0.83	12	10	108	90
Goat	120	3	2.5	11	9.17	1	0.83	-	-	2	1.67	-	-	1	0.83			18	15	102	85

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

Types of animals	No. of examined samples	wRBPT				wBAPAT				wRiv.T				wTAT			
		Positive		Negative		Positive		Negative		Positive		Negative		Positive		Negative	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Sheep	120	2	1.67	118	98.33	2	1.67	118	98.33	4	3.33	116	96.67	2	1.67	118	98.33
Goat	120	4	3.33	116	96.67	4	3.33	116	96.67	3	2.5	117	97.5	2	1.67	118	98.33