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## SERO-DIAGNOSIS OF BRUCELLOSIS IN COWS BY USING MILK

(With 2 Tables)

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التشخيص السيريولوجي لمرض البروسيلا في الأبقار بواسطة اللبن

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يعتبر اللبن من أكثر الاغذية عرضة للتلوث بالعديد من الميكروبات من مصادر مختلفة مما يجعله مصدرا لعدوى المستهلك بالكثير من الأمراض من أهمها حمى مالطة التي يسببها ميكروب البروسيلا. اشتملت هذه الدراسة على فحص عدد ٢٢٠ عينة لبن خام جمعت من مدينتى بنى سويف واهناسيا بمحافظة بنى سويف بواقع ١٧٠ عينة لبن خام جمعت من قطعان حلاب صغيرة و ٥٠ عينة لبن خام جمعت من بائعى التجزئه. تم فحص هذه العينات باختبار اللبن الحلقى كما تم فصل الشرش وفحصه باختبارات الروزبنجال والانتيجن الشريحي المحمض والتلبد الانبوبي والريفانول. اظهرت النتائج ان ١٤ (٨,٢%) ، ٢٠ (١١,٨%) ، ٢٤ (١٤,١%) ، ٤ (٢,٤%) و ٧ (٤,١%) من عينات قطعان الحلاب الصغيرة و ٣ (٦%) ، ٥ (١٠%) ، ٨ (١٦%) ، ١ (٢%) و صفر (صفر%) من عينات اللبن المجمع من بائعى التجزئة كانت ايجابية عند فحصها باختبارات اللبن الحلقى والروزبنجال والانتيجن الشريحي المحمض والتلبد الانبوبي والريفانول على التوالي. دلت النتائج على ان اللبن المفحوص من المدينتين يمثل خطورة على صحة الانسان وان له دور فعال في نقل العدوى للانسان كما تم مناقشة الاشتراطات الصحية الواجب اتباعها لوقاية وحماية الانسان من مرض البروسيلا.

### SUMMARY

A total of 220 raw milk samples were collected randomly from Beni-Suef and Ehnasia cities in Beni-Suef Governorate, comprising 170 small dairy herd and 50 retail milk samples. All the samples were examined serologically for brucellosis using MRT, wRBPT, w BAPAT, wTAT and w Riv.T. The results revealed that 14 (8.2%), 20 (11.8%), 24 (14.1%), 4 (2.4%) and 7 (4.1%) out of 170 small dairy herds milk samples were positive respectively. On the other hand, 3 (6%), 5 (10%), 8 (16%), 1 (2%) and 0 (0%) out of 50 retail milk samples were positive for MRT, wRBPT, w BAPAT, wTAT and w Riv.T respectively. The

results indicated that the milk of small dairy herds and retailers in both examined cities play a dangerous role in transmitting infection to human and constitute a public health hazard. The necessary measures to control the disease were discussed

*Key words: Brucellosis, milk, cows*

## INTRODUCTION

Brucellosis is a widely prevalent bacterial zoonotic disease especially in the Mediterranean area and developing countries. It is responsible for serious economic losses for dairy industry due to losses of calves, reduction in milk yield by 7-20%, some breeding troubles in infected animals and veterinary costs of diagnosis and preventive control measures (Sanders, 1989). Brucellosis is usually transmitted to humans either directly through contact with infected animals or their discharges or indirectly through ingestion of contaminated raw milk and dairy products prepared from unpasteurized milk (Sutra *et al.*, 1986 and Altuglu *et al.*, 2002).

Diagnosis of brucellosis is the corner stone for proper eradication of the disease. Isolation of the causative agent is still the land mark for proper diagnosis, but because of cost, time consuming, difficulty of performance, lack of sensitivity of most culture procedure and intermittent nature of its excretion in milk, the serological diagnosis is the main tool used for the detection of brucella infected animals. Although a wide range of serodiagnostic tests are available, there is no single test capable to identify the infected animals (Morgan *et al.*, 1969, Davies, 1971 and Salem *et al.*, 1987).

On the other hand, milk constitutes a highly desirable source of antibody for routine screening purposes and for the identification of infected individuals since sample collection is simple, not critical, less expensive, its antibody levels correlate with serum levels and single test can be applied to large numbers of cattle (Boraker *et al.*, 1981, Smith *et al.*, 1989 and Nielsen *et al.*, 1996).

In Egypt, milk is produced mainly by individual owners in small farms that lack of proper sanitary measures, which may be either consumed fresh, manufactured into dairy products or sell in retail markets. Therefore the present study is designed to declare the milk sero-prevalence and risk of brucellosis in Beni-Suef and Ehnasia cities in Beni-Suef governorate and to throw the light upon the public health significance and preventive measures to control brucellosis.

## MATERIALS and METHODS

### 1- Sampling:

A total of 220 raw milk samples were collected randomly from Beni-Suef and Ehnasia cities in Beni-Suef Governorate, comprising 170 small dairy herd milk samples (each sample contain the pooled milk of 3-5 animals) and 50 retail milk samples.

All samples were collected after thorough mixing without adding any preservatives and transferred directly to the laboratory in an insulating ice-box and kept in refrigerator until examination.

In case of small dairy herds all samples were collected from cows nearly in the mid lactation, free from subclinical mastitis and not receive any hormonal medication.

### 2- Preparation of whey:

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 donated from the Abassia Veterinary Research Institute, Cairo, Egypt, including:

- a- Milk ring test antigen.
- b- Rose Bengal plate test antigen.
- c- Buffered acidified plate antigen.
- d- Rivanol test antigen and Rivanol solution.
- e- Tube agglutination test antigen.

### 4- Methods:

Milk ring test (MRT), whey Rose Bengal plate test (wRBPT), whey Buffered acidified plate antigen test (wBAPAT), whey Tube agglutination test (wTAT) European method were carried out according to Alton, *et al.* (1988), while whey Rivanol test (wRiv.T) was performed according to National Veterinary Services Laboratories, Ames, Iowa, USA (1984).

## RESULTS

**Table 1:** Milk sero-prevalence of brucellosis in small dairy herd samples

	No of samples	Positives		Negatives		Suspicious	
		No	%	No	%	No	%
MRT	170	14	8.2	152	89.4	4	2.4
wRBPT		20	11.8	150	88.2	-	-
wBAPAT		24	14.1	146	85.9	-	-
wTAT		4	2.4	160	94.1	6	3.5
wRiv.T		7	4.1	163	95.9	-	-

**Table 2:** Milk sero-prevalence of brucellosis in retail milk samples

	No of samples	Positives		Negatives		Suspicious	
		No	%	No	%	No	%
MRT	50	3	6	42	84	5	10
wRBPT		5	10	45	90	-	-
wBAPAT		8	16	42	84	-	-
wTAT		1	2	49	98	0	0
wRiv.T		0	0	50	100	-	-

## DISCUSSION

Since brucellosis was first recorded in Egypt by Ahmed (1939), it remains an important public health and animal problem and received special attention in the last three decades due to higher incidence of brucellosis associated with cattle importation in open door policy for establishment of intensive breeding farms.

The results summarized in Table (1) revealed that 14 (8.2%), 20 (11.8%), 24 (14.1%), 4 (2.4%) and 7 (4.1%) out of 170 small dairy herds samples were positive for MRT, wRBPT, w BAPAT, wTAT and w Riv.T respectively.

On the basis of MRT nearly similar results were reported by Robertson, *et al.* (1980) and Abdel-Hakiem (1999), as they found MRT positive in 8.42 and 8% of milk samples respectively. Higher prevalence (11.4%) was recorded by Salem, *et al.* (1987), 10% by Hamdy (1992), 29.2% by El-Sheery (1993), 38.33% by Abdel-All (2001) and 12.38% by El-Bassiony, *et al.* (2007). On the other hand, lower prevalence (0.2%) was recorded by Awad, *et al.* (1977), 4% by Bastawrous (1987), 4.1% by Hosein and El-Kholy (1993), 4.31% by Gandara, *et al.* (1994) and 0.99% by Kadry (1996).

Several investigators referred to MRT as simple, rapid, accurate, highly sensitive, reliable and useful tool for detecting brucella agglutinins in milk of individual cows or herds (Ferguson and Robertson, 1954, Nicoletti and Burch, 1969, Morgan *et al.*, 1978, Boraker *et al.*, 1981, Salem *et al.*, 1987, El-Gibaly *et al.*, 1991, Hamdy (1992), Hosein and El-Khoy, 1993, Osman *et al.*, 1997, Abdel-Hakiem, 1999 and Bandara and Mahipala, 2002 ).

It also gave false positive reaction when the samples were taken near the end of lactation period, from mastitic milk quarters, shortly after parturition, from cows with hormonal disorders or when the colostrum included in the samples (Keer *et al.*, 1959, Bercovich and Moerman

1979, Corbel *et al.*, 1984 and Mac Millan, 1990), which are avoided during samples collection in this study.

Concerning the results of the wRBPT, lower values 4.7 and 4.29% were recorded by Abdel-Hakiem (1999) and El-Bassiony *et al.* (2007) respectively, while higher value (22.1%) was recorded by Abdel-Rahman (1991).

Regarding wBAPAT lower value (4.29%) was obtained by El-Bassiony *et al.* (2007), while higher value (38.9%) was recorded by Abdel-Rahman (1991).

In case of wTAT, nearly similar result (2.2%) was recorded by Turutoglu *et al.* (2003), while higher results (5.24 and 28.6%) were recorded by El-Bassiony *et al.* (2007) and Abdel-Rahman (1991) respectively. Moreover, nearly similar results to wRiv.T were recorded by Abdel-Hakiem (1999) (4%) and El-Bassiony *et al.* (2007) (4.29%).

Shifting to the results summarized in Table 2, 3 (6%), 5 (10%), 8 (16%), 1 (2%) and 0 (0%) out of 50 retail milk samples were positive for MRT, wRBPT, w BAPAT, wTAT and w Riv.T respectively.

The results reported herein are higher than those reported by Abd-Alla *et al.* (2000) in case of MRT and wRBPT, while lower in case of wRiv.T, as they reported 3.67, 3.53 and 2.86% with MRT, wRBPT and wRiv.T, respectively. On the other hand, Mishra (1982) and Arimi *et al.* (2005) pointed out higher prevalence of brucellosis (7 and 12.3% respectively) by using MRT.

There were some differences in the reactions from one test to another, which may be attributed to inability of some bovine antibodies subisotypes to be detected by all tests. IgG1, a very important subisotype, could be detected by RBPT, BAPAT and Riv.T but missed by TAT and MRT. IgG2, (relatively less important subisotype), could be detected by TAT, RBPT, BAPAT and Riv.T. IgM which is a cause of false positive, but its importance came from its presence at a very early stage of infection, IgM could be detected by MRT and TAT but not by RBPT and Riv.T (EL-Enbaawy *et al.*, 1995). IgA in milk does play an important role in the MRT, IgM also participates in this reaction (FAO/WHO, 1986). In addition to defatting process before the preparation of whey, removal of the solid part using rennin, the changes in pH, the changes in the molecular weight of immunoglobulins and most of the milk immunoglobulins are present on the surface of the fat globule (Sutra *et al.*, 1986, Hamdy, 1997 and Abdel-Hakiem, 1999).

In conclusion, this study showed that the milk of small dairy herds and retailers in both examined cities play a dangerous role in

transmitting infection to man, so efficient boiling or pasteurization of milk before consumption especially in infected areas to safeguard the consumers, urgent need for effective program for the control of this disease in reservoir animals in Egypt and educational programs to those sharing in milk production and handling as well as processing of dairy products. Further studies on brucellosis should be conducted in other areas for setting up priorities for control measures.

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