

Sero Diagnosis of Brucellosis by Using Simple and Rapid Field Tests with Emphasis on Some Possible Risk Factors in Humans

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Abstract: This study was designed to investigate sero prevalence of brucellosis and identify possible risk factors associated with human brucellosis. 400 animals (150 cows, 70 buffaloes, 80 goats and 100 sheep) were selected from veterinary clinics and abattoirs suspected to suffer from brucellosis from different localities in Kalyoubia governorate, as well as 280 persons suffering from fever suspected to be brucellosis were collected from fever hospitals at the same localities in Kalyoubia governorate. Serological tests were carried out by using mainly simple rapid field tests as Buffered Acidified plate Agglutination Test (BAPAT), Rose Bengal plate Agglutination test (RBPAT) and lateral Flow Assay (LFA). The results showed that the percentage of positive reactors was 6.5%, 6% and 5.5% by using BAPAT, RBPAT and LFA respectively in examined animals. Moreover, the occurrence of brucellosis was more in goats (7.5%) and sheep (6%) than in cows (4.7%) and buffaloes (4.3%). Regarding the results of examined persons, the percentage of positive reactors was (11.1%) by using RBPAT and (10.7%) using LFA respectively. The occurrence of brucellosis was high in males (11.6%) than females (8.9%), in rural (12.5%) than urban inhabitants (6.3%) and in occupations dealing with animals or its products (11.6%). From the possible risk factors, the majority of cases had a history of contact with infected animals (11.6%) and had no Knowledge about the disease (13.3%) and who eats ice cream bought from street vendors (16.7%) or soft cheese (11.4%). So knowledge of risk factors is a vital in control and prevention programmes. The public health importance as well as the suggested recommendations for prevention and control were discussed.

Key words: Brucellosis • Rapid field serodiagnosis • Risk factors in human • Kalyoubia governorate

INTRODUCTION

Brucellosis is an important Zoonotic disease of wild and domestic animals in which man is an accidental host. It has a world wide distribution, especially in Mediterranean countries and the Middle East and it remains a significant public health concern [1].

Brucellosis is still a serious disease problem facing the veterinary and medical professions due to appreciable economic losses to the livestock industry in infected areas resulting from abortions, sterility, decreased milk production and the cost of culling and replacement animals [2]. Additional losses result from human infection (Undulant fever) with its prolonged misery, debility and generalized aching, which may last for months or years

[3]. In human, consumption of contaminated food and occupational contact are the major risks of infection, traditional eating habits including the consumption of unpasteurized milk and fresh cheese and butter, is particularly common in the rural areas. These products are the primary causes of the spread of brucellosis [4]. Brucellosis has been an occupational risk for farmers, shepherds, butchers, laboratory workers and veterinarians as they are contracting the disease through inhalation of contaminated aerosols, contact with conjunctival mucosa, or entry of the bacteria through cuts and abrasions in the skin as a result of contact with infected animals or their products [5]. Contact with infected materials such as aborted feti, placenta, urine, manure and carcass has been reported in some countries to cause human brucellosis in

60-70% of cases [6]. Although isolation and identification is considered as gold standard for diagnosis but brucella culture takes several days and weeks and represents a great risk of infection for technicians, so a variety of serologic tests can be used for detection of brucella specific antibodies as Rose Bengal plate Antigen test (RBPAT), Buffered Acidified plate Antigen test (BAPAT) [7]. Recently, Immuno chromatographic brucella specific immunoglobulin lateral Flow assay (LFA) is a rapid, simplified test for the qualitative detection of specific antibodies in a variety of body fluids [8,9]. LFA is highly sensitive and specific and the application of it requires neither specific equipment, refrigerator, electricity, nor training, making this assay ideal for use in poor countries [10]. In order to prevent further transmission and spread of infection, using rapid tests is desirable. So the aim of this study was to investigate the sero prevalence of brucellosis among animals and humans at Kalyoubia governorate and to assess the possible risk factors associated with its occurrence to clarify its public health importance by using rapid screening field serological tests.

MATERIALS AND METHODS

Animal Samples: A total of 400 blood samples were collected from different animal species (150 cows, 70 buffaloes, 80 goats and 100 sheep). These animals were randomly selected from veterinary clinics, abattoirs and/or from small holder farms located in some villages of Kalyoubia governorate. All the examined animals were mature aged and were subjected to clinical and field investigation to collect some knowledge on their fertility status.

Human Samples: A total of 280 blood samples were collected from persons suspected to suffer from brucellosis based on history taking and clinical manifestations as fever attacks, sweating, back pain, chills, etc) from fever hospitals. Persons were interviewed using a standardized questionnaire which covered demographic data (Age, sex, occupation, residence and educational level) and other potential risk factors as contact with animals, mode of contact (cleaning farms, delivery or handling aborted and slaughtered animals), consumption of unboiled milk and milk products and general knowledge about route of transmission, awareness level and sanitation habits

Serological Examination: The blood samples collected

from all subjects (animals and humans) were centrifuged at 3000 r.p.m for 10 min to separate serum and each serum sample was labeled and stored at – 20°C until used. All sera were sent to the Animal Health Research Institute, " Brucella unit", Dokki, Giza, Egypt, to be examined by Buffered Acidified plate Antigen Test (BAPAT) and Rose Bengal plate Antigen Test (RBPAT) as described by [11] and by Lateral Flow Assay (LFA) according to the manufacturer's instructions and the test kits were obtained from Quiking Biotech Co. Ltd. China.

RESULTS AND DISCUSSION

In most developing countries, brucellosis is still an endemic disease in human and animals, so in order to control and eradicate the disease, it is very important to establish on appropriate serological methods which depends on the use of two or more tests and then use more specific test to confirm any positive cases. In the present study, serological investigation revealed that out of 400 examined farm animals from different species, 26 (6.5%) were positive for brucellosis using BAPAT, 24 (6%) using RBPAT and 22 (5.5%) using LFA. Concerning results of different serological tests among the examined animals, Table (1) indicated that the percentage of brucella positive reactors among cows, buffaloes, goats and sheep were 5.3 %, 4.3%, 10% and 7% respectively by using BAPAT, while by using RBPAT, it reached 4.7%, 4.3%, 8.8% and 7% respectively.

Moreover, the percentage of brucella positive reactors among them by using LFA was 4.7%, 4.3%, 7.5% and 6% respectively. These results were in accordance with [12-14] but lower than [10,15] and higher than [16]. Variations in the recorded results among examined farm animals could be due to the course of the disease, locality, rate of exposure to infection, reproductive status, in addition to the variety of the used diagnostic techniques. The higher rate of brucellosis in goats and sheep in the present study may be related to the nature of raising of these species as mobile flocks and always in movement all over the year for grazing and hence can be exposed to several routes of infection, moreover, the bad habits of farmers and shepherds in collecting and keeping both aborted, pregnant and non pregnant sheep and goats in the same flock and with lack of proper sanitary condition [17]. This explains why the rate of infection was higher in small ruminants than large one. It is worth to mention that where brucellosis exists in animals, the disease offers

Table 1: Results of serological tests for brucellosis among different animal species

Species	Examined No	BAPAT		RBPAT		LFA	
		Positive	%	Positive	%	Positive	%
Cows	150	8	5.3	7	4.7	7	4.7
Buffaloes	70	3	4.3	3	4.3	3	4.3
Goats	80	8	10	7	8.8	6	7.5
Sheep	100	7	7	7	7	6	6
Total	400	26	6.5	24	6	22	5.5

Table 2: Results of serological tests for brucellosis among examined persons

Examined No.	RBPAT		LFA	
	Positive	%	Positive	%
280	31	11.1	30	10.7

Table 3: Socio -demographic profile of examined persons by serology

Variable	Examined No.	Positive	%
Sex			
Male	190	22	11.6
Female	90	8	8.9
Total	280	30	10.7
Residence			
Rural	200	25	12.5
Urban	80	5	6.3
Total	280	30	10.7
Age			
≤ 15	30	3	10
16-25	70	9	12.9
26-35	75	9	12
36-45	65	7	10.8
≥46	40	2	5
Total	280	30	10.7
Occupation			
House wife	90	8	8.9
AAPH	190	22	11.6
Total	280	30	10.7
Education status			
Illiterate	90	10	11.1
Primary	30	3	10
Matriculation	80	9	11.3
Graduate and above	80	8	10
Total	280	30	10.7

AAPH= Animal and Animal Products Handlers

hazard to humans and serological tests appear to be the reliable and dependable tools in diagnosis. Serological investigations revealed that out of 280 examined persons, 31 (11.1%) were positive for brucellosis using RBPAT and 30 (10.7%) using LFA respectively. These results are in accordance with [18,19], but higher than [20].

From the previously mentioned results of serological tests among examined animals and human, it is evident that RBPAT detects lower number of infected animals

Table 4: Seropositivity of brucellosis in examined persons on the basis of different risk factors

Variable	Examined No.	Positive	%
Contact with animals			
Yes	190	22	11.6
No	90	8	8.9
Total	280	30	10.7
Eating habits			
Drinking raw milk	80	7	8.8
Eating ice cream	60	10	16.7
Eating soft cheese	70	8	11.4
Eating butter	70	5	7.1
Total	280	30	10.7
Knowledge About disease			
Yes	130	10	7.7
No	150	20	13.3
Total	280	30	10.7

than those detected by BAPAT. This could be attributed to the fact that the amount of serum used in BAPAT is greater than the amount of serum in RBPAT. Moreover the PH (3.65) of Rose Bengal antigen allows less amount of IgM to share in the reaction but final PH of BAPAT (4.2 ±0.04) permits the test to detect most classes of immunoglobulins (IgM, IgG1, IgG2 and IgA) in serum of infected animals. Although IgM is the first class of immunoglobulins appearing after infection, yet it was proved to be of non specific nature, Besides, most Gram negative bacteria produce IgM similar to those produced by Brucellae [11,21]. Moreover, RBPAT detects mainly IgM and IgG1. Despite these limitations, the RBPAT may be used as a screening test to ascertain exposure of animals to infection due to brucella species. So the conventional agglutination tests have good sensitivity but their lack of specificity and the occurrence of false positive make a specific test necessary. The least seroprevalence rate recorded with the LFA was indicative of its very high specificity and due to the high sensitivity, specificity and simplicity of the test and especially that the test does not involve any expertise nor refrigeration, it is recommended that this assay should be used for serological survey of brucellosis as a screening test, particularly in the rural areas [22]. The LFA has several practical advantages that allows testing on the spot and that may make it the method of choice when testing animals in remote areas or when testing animals from nomadic population. Practical advantages include that the assay is very simple to perform without the need for specific equipment, training, or electricity. Importantly, the assay gives a very clear result and is very easy to read by visual inspection for staining of a line in the test zone of the assay device. Furthermore, the assay components are highly stable and

well standardized which for instance is not the case with the antigen used in RBPAT that requires careful titration and the devices can be stored without need for refrigeration [10,23]. The LFA is more sensitive and specific which allow its uses as a confirmatory test in all cases which suspect the presence of brucellosis. So RBPAT is sensitive but LFA is more specific and its speed makes it available for rapid presumptive test which can replace RBPAT in brucellosis control programs. By using the LFA as a field test, the identification and tracing of animals and their owners is much less problematic and intervening measures to control the disease could be started without delay with less risk of further transmission and spread of infection. Regarding the potential risk factors of the studied human cases, it is evident from this study that there was a predominance of male (11.6%) over female distribution (8.9%). This is in accordance with studies conducted by Afifi *et al.* [24] and Jennings *et al.* [25]. The higher rate of sero prevalence of brucellosis in males compared to females was probably due to increase involvement of men in farming domestic animals and handling their products in rural areas [4]. In addition, it may be due to lack of awareness among females or less number of females participating in potentially dangerous activities like handling dystocia cases [26].

Concerning the residence, the occurrence of brucellosis was higher in rural areas (12.5%) as compared to urban areas (6.3%). This can be associated with increased human-animal interaction in rural areas and consumption of local dairy products [21,27]. However, [24] showed similar distribution in both localities in all parts of Egypt whereas, animal exposure can occur in all regions and un-pasteurized dairy products are widely available throughout country.

Brucellosis affects all age groups but in this study, the age group 16-25 years and 26-35 years were the most commonly affected 12.9% and 12% respectively and this may be attributed to that the majority of the workers engaged in veterinary care, rearing, milking are adults. This is agree with Alumneef *et al.* [28] who stated that brucellosis is mostly often affect adults but with a low incidence among children and the elder. In addition to, the occupations dealing with animals or its products formed (11.6%) of the positive cases. This is comparable with Bikas *et al.* [29] who found that 85.7% of cases were animal breeders and Minas *et al.* [30] detected that people in occupations dealing with animals constituted the majority (91.5%) of patients. These occupations included farmers, slaughter house workers, veterinarians, shepherds, butchers as well household members as they

often help their family in flock management and acquired the infection by direct contact with animals. Concerning to educational status, the proportion of sero positivity ranged from 10% in primary and graduate level education to 11.3% and 11.1% in those workers having education of matriculation and illiterate, so there is no relationship between workers educational level and their immune status. The results are similar to that recorded by Sumer *et al.* [31], However Karimi *et al.* [32] have reported opposing results, they have shown a strong positive correlation between low literacy and sero positivity. From this study, it is evident from (table 4) that direct contact with domestic animals and their products specially sheep and goats was an important risk factor for brucellosis and represented 11.6%.

As regard eating habits, frequency of eating ice cream from street vendors and eating soft cheese, drinking raw milk, eating of butter were associated with brucellosis and represented 16.7%, 11.4%, 8.8% and 7.1% respectively. Eating ice cream from street vendors was an important source of infection in this study because of its unknown source and possibly made from the milk of infected animals. Moreover, soft cheese made using traditional methods which do not ensure killing organism has also been implicated as a source of infection. Drinking of unboiled milk also appeared to have an association with brucellosis, this was in accordance with Meky *et al.* [3] and Yohannes and Gill [26] but disagreed with Serra *et al.* [33] who demonstrated that there is no statistically significant relationship between brucellosis and consumption of unboiled milk. This may be due to the fact that drinking fresh milk without boiling is an uncommon practice as owing to fear of contracting other infection such as tuberculosis. Regarding, Knowledge about the disease among the studied group, there was (13.3%) didn't have any knowledge about brucellosis, its mode of transmission and clinical symptoms thus, the lack of awareness about the disease is considered as the main risk factor for brucellosis.

The study concludes that brucellosis is a disease of public health importance in Egypt, especially at rural areas and emphasizes the importance of contact with infected animals and their products and ingestion of local dairy products as methods of transmission of brucellosis. This means that prevention of brucellosis in human is dependant on control of the disease in domestic livestock. This can be achieved by elimination of infected animals and mass vaccination of healthy ones. Also, it could be concluded that LFA could be ideal as a field rapid screening test for developing countries and rural settings,

suitable for large- scale screening or presumptive test. Moreover, the high sensitivity and specificity of LFA allows its use as a confirmatory test in combination with RBPAT as a screening assay. Knowledge of risk factors is a vital in control and prevention programmes. Thus, an extension education campaign, particularly in high risk area could aid in decreasing the incidence of brucellosis. A control programme for human brucellosis would depend on a large extent on public health education about the disease and its risk factors as the use of protective clothing while handling still- births or products of conception can reduce occupation related disease and the avoidance of unpasteurized dairy products will prevent infection in the general population and there must be good administrative arrangement and ensuring the maximum cooperation of health and veterinary authorities and alertness of the physicians to include brucellosis in their immediate diagnosis especially in the high risk groups. So to deal with brucellosis, awareness on risk factors must be part of extension education campaign. Besides, regular surveillance of the disease needs to be integrated into control and prevention programme at local and national level.

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