

Environmental study about milk source for causes brucellosis

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Abstract:

Objective:

Brucellosis is possess a significant public health problem in Iraq. In this study we diagnosis of brucellosis from infected animals for brucellosis by serological method (Milk Ring test) and also used culture method in diagnosis of animal brucellosis. A total of 87 milk samples were collected from animals with high suspected brucellosis. When culture was applied to milk samples, 6 cases were positive for Brucella species but 9 cases were positive for milk ring test.

Duration and place of study: Milk samples were obtained from infected animals for brucellosis, referred to different city in Baghdad and another province like: Al-Thawra city, Abu Ghraib, Al-Habebia, Al-Yosfia, Al-Fudhaliyah and different province in Iraq, which include: Karbala, Diyala and Ninawa, as well as access to statistics and maps of the provinces of Iraq from Ministry of Agriculture/Veterinary Directorate, during the duration from (March to December 2014).

Methodology: A total 87 milk samples were collected from infected animals. The diagnosis of brucellosis was established by clinical findings and serological test (Milk ring test and then confirmed by culture method and biochemical test).

Results: A total of 9 of 87 sample (10.34%) samples were positive result by MRT and 6 of 87 sample (6.89%) samples were positive result by culture was applied to animal's milk.

Conclusions: These results indicate that infected livestock for Brucella like: sheep, goat, cow and buffalo located in epidemiological regions in Iraq especially in Baghdad province across the study period and showed milk culture is the gold standard for diagnosis of brucellosis compared with serological methods (Milk Ring test) for the diagnosis of brucellosis.

Key Words: Environmental, Brucellosis, Milk Ring test, Culture, Livestock.

دراسة بيئية عن مصادر الحليب المسببة لداء البروسيلة

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الخلاصة:

الهدف: يشكل داء البروسيلة مشكلة صحية عامة في العراق في هذه الدراسة نحن شخصنا داء البروسيلة في الحيوانات المصابة بالبروسيلة بواسطة الطريقة المصلية (إختبار حلقة الحليب) وكذلك إستخدامنا طريقة الزرع في تشخيص داء البروسيلة في الحيوانات. من مجموع ٨٧ عينة حليب جمعت من الحيوانات المحتمل إصابتهم بداء البروسيلة. أظهرت النتائج بأستخدام طريقة زرع عينات الحليب إن ٦ حالات كانت إيجابية للفحص ولكن ٩ حالات كانت إيجابية لأختبار حلقة الحليب.

مدة ومكان الدراسة: أخذت عينات الحليب من الحيوانات المصابة بداء البروسيلة، والحصول عليها من مدن مختلفة في محافظة بغداد والمحافظات الأخرى مثل: كربلاء، ديالى ونيوى، كذلك الحصول على الأحصائيات والخرائط لمحافظات العراق من وزارة الزراعة / دائرة البيطرة ، للمدة من شهر آذار لغاية كانون الأول ٢٠١٤.

طريقة العمل: أجريت الدراسة على 87 عينة حليب مأخوذة من الحيوانات المصابة بداء البروسيلة. إعتد التشخيص على العلامات السريرية المؤكدة بواسطة إختبار حلقة الحليب، وطريقة الزرع، وتأكيداها بواسطة صبغة كرام ومختلف الأختبارات الكيميائية.

النتائج: تم الحصول على 9 (١٠.٣٤%) عينة موجبة لأختبار حلقة الحليب وكذلك الحصول على 6 (٦.٨٩%) عينة إيجابية للزرع والمأخوذة من حليب الحيوانات المصابة.

الأستنتاجات: النتائج تشير إلى إن الحيوانات المصابة بالبروسيلة مثل: الأغنام، الماعز، الأبقار والجاموس الموجودة بالمناطق الوبائية في العراق وخصوصاً في محافظة بغداد خلال فترة الدراسة ، ولاحظنا إن طريقة زرع الحليب هي الطريقة الذهبية لتشخيص البروسيلة مقارنة مع الطريقة المصلية (إختبار حلقة الحليب) في تشخيص داء البروسيلة.

الكلمات المفتاحية: البيئة، داء البروسيلة، المرضى، إختبار حلقة الحليب، الزرع، الماشية.

Introduction:

Brucellosis is the most widespread zoonosis in the world and the infection is almost invariably transmitted to people by direct or indirect contact with infected animals or their products. The annual occurrence of human brucellosis in the world is more than 500,000 cases [1, 2]. The most commonly affected animals are cows (*B. abortus*), sheep and goats (*B. melitensis*), pigs (*B. suis*), and some other domestic animals. It causes abortion and infertility in animals [3, 4] and undulant fever in man [5].

Human and animal health is closely linked and people depend on animals for nutrition, companionship and socio-economic development. The route of transmission of animal diseases, including zoonoses, can be direct or indirect. Examples of direct or indirect transmission are via contact with infected animals, animal products such as contaminated food, by consumption of contaminated drinking water or by vectors that carry infection between hosts. Possible vectors are ticks, flies, mosquitoes, fleas and lice. Studies indicate⁵ that about 40% of all human

infectious diseases are transmitted from animals. About 75% of newly emerged diseases the last decade have been transmitted from animals or animal products [6].

Cattle are most often infected by *Brucella abortus*, although *B. melitensis* and rarely *B. suis* can also infect cattle. Outbreaks of bovine brucellosis are associated with abortion during late gestation (third trimester), weak newborn calves, retained placenta, metritis, orchitis, reduced milk production and infertility in cows and bulls. The outcome of the disease is dependent on virulence of the infective strain, age, reproductive and immunological status and route of infection [7]. Vaccination of cattle with *B. abortus* strain 19 or RB 51 can greatly reduce the susceptibility for infection of the homologous species [8, 9].

There are many serological tests used for diagnosis like Rose Bengal test, Standard Agglutination test [10, 11] and MRT has been used for many years for detection of dairy cows infected with *B. abortus*, since milk constitutes a highly desirable source of antibody for routine screening purpose and for the identification of infected individuals as sample collection is simple and noninvasive [12-14]. Blood culture provides definite proof of brucellosis but may not provide a positive result for all patients. Lysis centrifugation and blood clot culture techniques have yielded encouraging results in recent reports [15, 16] in terms of sensitivity and rapidity. The modern automated blood culture systems have somewhat improved the speed of detection.

The aim of the study was to as say the incidence of brucellosis in animals by using serological tests, which include: - Milk Ring test and isolation for detection of *Brucella* antibody in milk samples.

Materials and Methods:

A total of 45 milk specimens were collected from infected animals, referred to different region in Baghdad and different province in Iraq, during the period from March to December 2014. The diagnosis of brucellosis was established by the presence of a compatible clinical picture [17] including abortion fetus or infected new born and serological diagnosis was carried by positive Milk Ring test and also used culture method, moreover demographic, occupational, clinical, and risk factor details were recorded for animals.

The Statistical Analysis System- SAS [18] was used to effect of different factors in study parameters. Chi-square test was used as a test to compare between percentages in this study.

Serological tests:

- Milk ring test (MRT):-

The Milk Ring test method was used for the detection of *Brucella* antibody in milk samples. This technique is very easy to perform it especially in dairy herds. The principle of agglutination between antibodies contained in milk and dye colored bacterial antigen brucellosis to form antigen-antibody complex that are progressively carried by the fat towards the surface of the milk and formed a blue violet ring [19].

Traditional test:

- Culture and biochemical test:-

All media were prepared according to the manufacturing company instructions; *Brucella* agar or Trypticase soy agar were used sterilized by autoclaving at 121°C for 15 min, after cooling the media to 56 °C, they were brought to antibiotics with 5% of fetal calf serum for *Brucella* nutrition and mixture with media [20] and put in petri dish. Otherwise the media were incubated at 37 °C for 24 hours to ensure sterility. On the other hand, they were brought media (Blood agar) and brought Trypticase soy broth were prepared according to the manufacturers company instructions; and then sterilized by autoclaving at 121 °C for 15 min.

The first part of the milk was inoculated into: Blood agar, *Brucella* agar, trypticase soya agar and trypticase soya broth culture medium containing both a solid and a liquid phase [21]. Then it was subculture on duplicate agar plates and incubated one in air and the other in an atmosphere at 37°C in the presence of 5-10% CO₂. After 7-30 days, colonies grown in the solid phase were identified by inoculation into *Brucella* agar or trypticase soya agar and taken the growth of colonies by loop and spreaded on the surface of plates containing blood agar media and performance of biochemical tests [22].

Results:

A total of 87 milk specimens have been collected from animals. The diagnosis of brucellosis was established by abortion fetus or infected new born and used serological test like:- Milk Ring test and used conventional culture and different biochemical test. The main serological test used for diagnosis of brucellosis was the Milk Ring test (MRT), total of 87 samples, 9 (10.34%) samples were positive for MRT. (Table 1).

Table 1: Relation between the different animals using MRT (Positive & Negative).

No.	Type of animals	Province	Number of sample	Positive		Negative		Chi-square χ^2	
				Sample	%	Sample	%		
1	Buffalo	Baghdad	24	3	12.50	21	87.5	13.95 **	
2	Buffalo	Ninawa	11	5	45.45	6	54.54	4.18 *	
3	Cow	Baghdad	9	0	0	9	100	15.00 **	
4	Cow	Karbala	9	1	11.11	8	88.88	14.51 **	
5	Cow	Diyala	4	0	0	4	100	15.00 **	
6	Goat	Baghdad	2	0	0	2	100	15.00 **	
7	Goat	Ninawa	5	0	0	5	100	15.00 **	
8	Sheep	Baghdad	18	0	0	18	100	15.00 **	
9	Sheep	Ninawa	5	0	0	5	100	15.00 **	
Total			4	4	87	9	0	78	-

** (P<0.01) = highly significant, ns: non-significant.

Out of 87 milk samples were detected by MRT revealed 9 (10.34%) positive, whereas 6 (6.89%) samples were positive using conventional culture. (Table 2 & Figure 1).

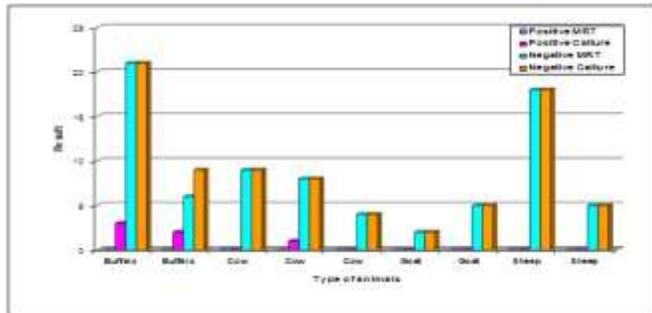


Figure1: Comparison of *Brucella* antibody titer (MRT) and conventional culture Result.

The genus characterization were performed using Gram staining and identification by different biochemical tests. (Table 2).

Table 2: Biochemical Characters of *Brucella* Isolates.

No.	Name of Tests	Isolates
1.	Oxidase	-
2.	Catalase	-
3.	Urease test	-
4.	Indole test	-
5.	Motility	-
6.	Production of H ₂ S	-

+ = Positive, - = Negative

In the present study evidence of the severity and incidence of *Brucella* in Iraq, explaining maps and

statistics from Ministry of Agriculture / Veterinary Directorate. (Table 3) and (Figure 2 & 3) [23].

Table 3: The scores concerted *Brucella* for different years in Iraq provinces.

Province	2005	2006	2007	2008	2009	2010	2011	2012	2013
Ninawa	1699	1425	51	14	30	231	135	139	102
Kirkuk	11344	405		8	51	473	62	35	24
Salahuddin	3483	150	1150	30	610	675	2654	14	17
Al-Anbar	286	423	200	871	0	0	596	0	0
Diyala	1736	3880	240	0	35	71	328	107	6
Wasit	14426	1869	460	120	100	97	243	130	35
Baghdad	1056	203	873	67	27	34	71	112	47
Babil	55	6850	195	120	766	637	397	102	48
Karbala	0	71	22	0	0	0	180	19	10
Al-Najaf	0	5	0	0	0	480	200	0	0
Al-Devanya	40	1858	0	0	0	185	0	175	10
Al-Muthana	0	336	231	0	0	0	0	0	0
Dhiqar	0	0	0	5	10	111	69	16	16
Mayaan	1041	2553	974	44	120	0	34	6	0
Al-Baira	0	255	21	0	0	129	120	62	7
Total	35157	20283	4330	1079	1749	3123	5000	908	322



Figure 2: Map for Brucellosis infected in bovine concerted in all Iraq provinces.

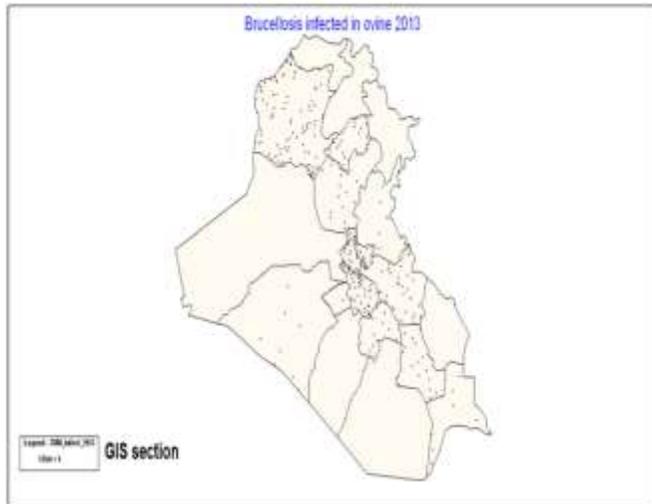


Figure 3: Map for Brucellosis infected in ovine concerted in all Iraq provinces.

Discussion:

Brucellosis is essentially a disease of animals, especially domesticated livestock, caused by bacteria of the *Brucella* group with humans as an accidental host. In other words it is a zoonosis. Brucellosis during the course of pregnancy carries the risk of spontaneous abortion or intrauterine transmission to the infant. Abortion is a frequent complication of brucellosis in animals, where placental localization is believed to be associated with erythritol, a growth stimulant for *B. abortus*. Although erythritol is not present in human placental tissue, *Brucella* bacteremia can result in abortion, especially during the early trimesters. Whether the rate of abortions from brucellosis exceeds rates associated with bacteremia from other bacterial causes is unclear. In any event, prompt diagnosis and treatment of brucellosis during pregnancy can be lifesaving for the fetus [24].

Milk Ring Test (MRT) has been used for many years for detection of dairy cows infected with *B. abortus*, since milk constitutes a highly desirable source of antibody for routine screening purpose and for the identification of infected individuals as sample collection is simple and noninvasive [25-26]. Statistical analysis showed that 9 (10.34%) animals revealed a positive result by MRT and 78 (89.65%) animals were negative result for MRT out of 87 animals occur in the different region in Baghdad and another province. Milk Ring Test (MRT) performed on bulk milk samples is

effective for screening and monitoring dairy cattle for brucellosis, but is less reliable in large herds. The test is reasonably sensitive but may fail to detect a small number of infected animals within a large herd and also some people used milk product from animal breeder.

Bacterial culture of milk samples used to identify the organism as a gold standard and should be used as the confirmatory diagnostic test. Taking the microbiological culture as a reference, 6 (6.89%) samples were positive by culture and 81 (93.10%) samples were negative out of 87 animal's milk samples and used different biochemical test and gram stain with positive culture for confirmation of the culture technique.

It is well known that unequivocal diagnosis of brucellosis requires isolation of the causal agent [27]. Although a presumptive diagnosis of brucellosis can be made by demonstrating high or rising antibody titers to *Brucella* antigens, isolation of the organism from blood, bone marrow, or tissue cultures is the only irrefutable proof of the disease [30]. Although *Brucella* can be isolated from cerebro spinal fluid, wounds, etc. In veterinary field, however, it is used as a diagnostic test in cattle and swine populations where incidence of brucellosis is relatively high and, as a screen procedure in low incidence areas. Anti-*Brucella* antibodies testing has a high diagnostic value on a tentative diagnosis made on the basis of case history and clinical findings.

In this study we are show statistically analysis for all Iraq provinces and we have also show a different infected in those provinces and specific years because we are show the brucellosis wide spread in Iraq not in Baghdad only so we was used different maps in Iraq provinces .

Finally, in this study we used serological test like MRT and used culture method for detection brucellosis in milk samples, so show culture is very important for diagnosis animal milk samples in comparison with serological test (MRT). We have also noted that culture needs a long time for the diagnosis of brucellosis, but it for accurate for the serological test.

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