IJBPAS, September, 2018, 7(9): 1786-1802
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'
www.ijbpas.com

THE UTILITY OF MRT TO SCREEN BRUCELLOSIS AMONG EWE AND NANNY GOATS MILK IN ERBIL GOVERNORATE / KURDISTAN REGION / IRAQ

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Received 14th March. 2018; Revised 9th April 2018; Accepted 14th May 2018; Available online 1st Sept. 2018

https://doi.org/10.31032/IJBPAS/2018/7.9.4551

ABSTRACT

The present work was undertaken to monitoring Brucellosis among ewes and nanny goats milk by using of Milk Ring Test. A total of 350 raw milk samples were collected during January 2017 to June 2017, these included 185 samples from ewes and 165 nanny goats. The overall prevalence of Brucella antibodies in all milk samples was 34 /350 (9.7 %). The highest rate was found in nanny goats milk 19/165 (11.5 %), while the lowest rate was from the ewe's milk 15/185 (8.1 %).

Out of 350 milk samples, only 29 (8.3 %) Brucella isolates were found, (6.5%) from ewes milk and (10.3 %) from nanny goats.

Relation between the result of MRT and isolation of Brucella species from milk indicated that (8.1%) samples from ewes were positive according to MRT compared with (6.5 %) samples gave isolates of Brucella species , and (11.5 %) from nanny goats was positive according to MRT compared with (10.3 %) samples gave isolates. Also, our result confirmed that (41.7 %) and (17.6 %) of isolates were Brucella abortus, while (58.3 %) and (82.4 %) were Brucella melitensis isolated from ewes and nanny goats milk consecutively.

The highest rate of frequency of Brucella antibodies according to MRT was found in April (15.4 %), then in March (10.9 %), while the lowest rate was found in May and June (7.7 %) for each month.

This study clarified that Brucellosis is still one of a significant public health hazard for the Kurdistan region. We recommend that MRT participate in the monitoring of Brucellosis in milk, and the consumers need to be accurately heated the milk to destroy this foodborne pathogen.

Keywords: Utility, Milk Ring Test, Brucellosis, Ewe Milk, Nanny goats milk, Erbil Governorate, Kurdistan Region, Iraq

INTRODUCTION

Brucellosis is an international bacterial zoonotic infection that transmissible to humans and a wide range of domestic and wild animals particularly food-producing animals including sheep, goats, cattle, camels, buffaloes, pigs, and reindeer. During the last two decades, the infection has also been identified in marine mammals, including Dolphins, beaked whales, Porpoises, cetaceans, and Seals, which may present an emerging hazard to persons occupationally exposed to infected tissues from them(1, 2).

Brucellosis is a foodborne and occupational zoonosis, so it distinguishes a public health trouble and one of the main causes of high morbidity and mortality. It is also a major cause of direct cost-effective losses resulting from clinical sickness, abortion, neonatal losses, reduced productiveness, decline milk production. Brucella disease is accountable for up to 20 – 25 % drop off in milk production, 10 – 15 % in meat production, 15 % loss of calves due to abortions, 30% increase in the average of animal substitution, and increased calving period of to 11.5 to 20 months in domestic animals, it is also a major hindrance for international trade of milk, meat, and their products (3,4).

Human brucellosis is a severely debilitating and disabling disease, it represents a high public health hazard, and poses a major threat to human health. It is a very old zoonotic disease and a recent indication from Egyptian ancient skeletons has revealed that this disease has been present for no less than 750 BC. Brucellosis is an extremely emerging infectious disease (EID) and one of the most significant reemerging zoonoses in several countries , also the wide map of human brucellosis has extremely converted

over the past decade, due to a range of complex factors of changing conditions such as lack of various hygienic conditions, the level of socio-economic behaviors, and governmental causes, collectively with increased globalization of peoples , animals , Birds , and the moving of food products around the world (5,6).

After the entrance of the Brucella to the human body, three major stages can be differentiated; incubation phase, acute phase, and the chronic phase. The incubation phase can be changeable and not easy to determine, but is normally two to four weeks, with a range of five days to five months. The acute phase is characterized by the onset of symptoms and signs like fatigue, fever, sweats, splenomegaly, and hepatomegaly. *Brucella* has numerous virulence factors, so it able to survive in host cells and avoid host immune responses to establish chronic foci of infection. Clinical polymorphism is very common and for this cause, brucellosis is frequently unrecognized in primary health care surroundings. Brucellosis is moreover recognized to cause severe clinical complications with the involvement of the internal organs, including sacroiliitis, osteomyelitis, spondylitis, peripheral arthritis**,** hepatic abscesses**,** bronchopneumonia, epididymitis, orchitis**,** meningitis, encephalitis, prostatitis, and Cardiovascular Complications(7, 8).

Brucellosis is an important human disease found around the world, particularly Mediterranean basin countries, the Middle East including Iraq and Iraqi Kurdistan, Arabian Gulf, Indian subcontinent, Countries of Europe, Africa, Asia, Central and South Americas, Mexico, and yet it is often unrecognized and frequently goes unreported. There are a few countries in the world that are officially free of brucellosis such as Australia, Canada, Japan, Cyprus, Denmark, Finland, The Netherlands, New Zealand, Norway, Sweden and the United Kingdom, even though cases until now occur in people of these countries returning from an endemic region(9, 10).

A high occurrence of Brucellosis in definite geographic regions is well documented, although broadly undervalued. It infects annually approximately 500,000 humans worldwide. Several investigators reported that this figure underestimates the proportion of the problem, and some of them evaluated that the figure of human Brucellosis situation may be up to 26 times

upper than the figure mentioned above (11, 12).

The disease is caused by various types of the genus Brucella, which favored to be host-specific. An infectious dose of 10–100 organisms is sufficient to cause systemic infection. *Brucella species* are facultative intracellular, Gram-negative coccobacilli (GNCB), very small (0.5–0.7 μm × 0.6–1.5 μm), with straight or slightly convex in shape and rounded ends, lack capsules or flagella and, therefore, are non-motile, nonspore forming, encapsulate. They are aerobic, but some strains require 5–10% carbon dioxide for primary isolation. Growth *in vitro* is slow and primary isolation may require 4 weeks incubation, grow at 37°C on Brucella agar, Albumin agar, Trypticase soy agar media, growth may be improved by the addition of serum or blood. Colonies appear transparent, raised, convex with an entire edge and a smooth, shiny surface on transparent media after 4-5 days of incubation, the primary culture of Brucellae reveals punctuate, non-pigmented, and-hemolytic colonies. Biochemically, carbohydrates are fermented without gas and acid (13, 14).

Until now, there are twelve different *Brucella species* have been described, each species may infect diverse host type, but each *Brucella species* has a favorite for its host type. Six traditional out of twelve species include Br.*abortus, Br. melitensis, Br. suis, Br. neotomae, Br. ovis, Br. canis* and six new species of *Brucella* include *Br. ceti, Br. pinnipedialis, Br. microti, Br. inopinata, Br. papionis* , *Br.vulpis* (15 , 16).

In recent years, 17 declared that the incidence of human Brucellosis in Iraqi Kurdistan is still upper than reported from adjacent nations, and Brucella infection has been recorded from all three Iraqi Kurdistan provinces. He pointed out that the incidence proportion in Erbil city was 10.7% in 2012, in Dohuk was 6.36% in 2011, and 976 cases were registered in Sulaimani province in 2013.

The consumption of contaminated milk and dairy products has been broadly authenticated as an essential way of Brucella transmission. particularly, unpasteurized milk or dairy products from infected ewes, nanny goats, and cow have been regarded as a source of infection for the inhabitants, mainly in developing regions, and the problem is increased when we know that an infectious dose of 10–100 organisms are sufficient to cause systemic infection. Therefore the objectives of this work were to

looking for the occurrence of Brucella antibodies and *Brucella species* among ewes and nanny goats milk in Erbil Governorate, to determine the sensitivity and specificity of MRT, and to study the relationship between the incidence of Brucella antibodies in ewes and nanny goats milk with months during the period of study. Also high lights on the risk of Brucellosis help in the understanding of milk and dairy products responsibility in the spreading of this disease, and to awareness on the significance of milk heat treatment.

MATERIALS AND METHODS

1- Study Design and Sampling

2- Three hundred and fifty (350) raw milk samples were collected among villages around Erbil city, during the period from January 2017 to June 2017. These included 185 samples from randomly selected lactating ewes and 165 samples from nanny goats. The milk samples were collected under sterile hygienic conditions according to (18). Each sample was collected into a sterile 10 ml plastic cup with a screw lid. The samples were aseptically transported to the Knowledge University /College of Science/ Department of pathological Analysis / Erbil City.

3- Observation of Brucella antibodies

In the laboratory, watching of Brucella antibodies in milk was done by using Milk Ring Test (MRT). The test was performed by adding one drop (0.03 ml) of MRT antigen to 1 ml of whole milk in a narrow test tube (11 x 100 mm). The milk and antigen mixtures were incubated at 37°C for 1- 3 hours. If the specific antibody is present in the milk it will bind to the antigen and rise with the cream to form a blue ring above the white milk column was considered positive. The test was interpreted as negative if the color of the Ring white and Column blue (18).

4- Isolation and Identification of Brucella

The isolation of *Brucella* from milk samples was done under sterile conditions at the Microbiology Laboratory, Pathological Analysis Department, *following standard procedures* (19). Plates were inoculated with milk and incubated aerobically and in the presence of 5%–10% carbon dioxide at 37°C. The plates were checked for up to 10 days for the presence of bacterial growth. The identification of *Br. abortus and Br. melitensis* were confirmed by Biochemical analysis (4).

5- Statistical analysis

Data were analyzed using the Chi-Square test and SPSS software version 15.

6- Sensitivity and Specificity of MRT

The sensitivity and specificity of MRT were calculated, using the bacterial isolation diagnostic method as a gold standard.

RESULTS

The overall prevalence of Brucella antibodies in ewes and nanny goats milk samples were 34 / 350 (9.7%). The highest rate of prevalence of Brucella antibodies was found in nanny goats milk samples 19/165 (11.5%), while the lowest rate of prevalence was from the ewes milk samples 15/185 (8.1 %) (Table1).

From Table 2, we showed that among 350 samples of ewes and nanny goats milk, only 29 (8.3 %) Brucella isolates were found. This result includes 12 (6.5 %) positive samples from ewe milk and 17 (10.3 %) positive samples from nanny goats milk.

When we study the relation between the result of MRT and isolation of *Brucella species* from ewe and nanny goats milk, we found that 15 / 185 (8.1 %) and 19 /165 (11.5 %) milk samples from ewes and nanny goats were positive according to MRT, compared with 12/185 (6.5 %) and 17 (10.3 %) samples gave isolates of Brucella species consecutively (Table 3).

Depending on Phenotypic features of *Brucella abortus* and *Brucella melitensis* isolated from ewe and nanny goats milk, we attained that 5 / 12 (41.7%) and 3 / 17 (17.6 %) of isolates were Brucella abortus , while 7 / 12 (58.3%) and 14 / 17 (82.4%) were Brucella melitensis respectively (Table 4).

Table 5 clarify that the relation between months and occurrence of Brucella antibodies in ewe and nanny goats milk samples during the period of study. From this table we observed that the highest rate of frequency of Brucella antibodies according to MRT was found in April 10 /65(15.4%), then in March 6 / 55 (10.9%), while the lowest rate was found in May and June 5/ 65 (7.7 %) for each month.

Table (1):- Prevalence of Brucella antibodies among Ewes and Nanny goats milk according to MRT

Type of Milk	No.Tested	Positive samples		Negative samples		Chi-Square	P value
		No.	%	No.	%		
Ewe	185	15	8.1	170	91.9	129.86	0.00
Nannygoat	165	19	11.5	146	88.5	97.75	0.00
Total	350	34	9.7	316	90.3	227.21	0.00

Table (2):- Isolation of Brucella species from Ewes and Nanny goats milk

Type of milk	No. Tested	+ive Isolation No.	%	-ive isolation No.	%	Chi-Square	P value
Ewes milk	185	12	6.5	173	93.5	140.11	0.00
Nanny goats milk	165	17	10.3	148	89.7	104.01	0.00
Total	350	29	8.3	321	91.7	243.61	0.00

Table (3):- The Relation Between Result of MRT and Isolation of Brucella species from Ewes and Nanny goats Milk

Type of milk	No. tested	Result of MRT No.	%	Isolation of Brucella No.	%	Sensitivity	Specificit
Ewe	185	15	8.1	12	6.5	80.0 %	93.5%
Nanny goat	165	19	11.5	17	10.3	89.5%	89.7%
Total	350	34	9.7	29	8.3	85.3%	91.7%

Table (4): Prevalence of Brucella species in Ewes and Nanny Goats Milk

Type of milk	No. Isolated	Br. abortus No.	%	Br. melitensis No.	%	Chi-Square	P Value
Ewes	12	5	41.7	7	58.3	0.33	0.56
Nanny goats	17	3	17.6	14	82.4	7.12	0.008
Total	29	8	27.6	21	72.4	5.83	0.016

Table (5): Relation between Months and Prevalence of Brucella antibodies (MRT) during the period from January 2018 – July 2018

Month	Ewes milk Examined positive	Nanny goats milk	Total examined samples	Total positive No.	%	Chi Square	P Value
January	2 /25	2 / 25	50	4	8.0	35.28	0.00
February	1 /25	3/ 25	50	4	8.0	35.28	0.00
March	3 /30	3/ 25	55	6	10.9	33.62	0.00
April	4 / 35	6 / 30	65	10	15.4	31.15	0.00
May	2 /35	3 / 30	65	5	7.7	46.54	0.00
June	3/35	2 / 30	65	5	7.7	46.54	0.00
Total	15 / 185	19 / 165	350	34	9.7	227.21	0.00

DISCUSSION

Brucellosis is mainly a disease of cattle, Buffalo, Camels, sheep, goats, and swine, and the transmission to humans occurs in several ways. Foodborne transmission is the most common route in which people become infected and results from the consumption of raw milk and other dairies products and raw or insufficiently cooked meat from infected animals. Transmission also occurs through skin wounds or mucous membranes, following direct contact with blood, urine, tissues, vaginal discharges, aborted fetuses or placenta, and during inhalation of airborne agents in an environment such as laboratories and slaughterhouses. Accidental inoculation of live vaccines, such as *Br. melitensis* Rev 1 and *Br. abortus* strain 19, can also occur, resulting in human infections. Human-to-human transmission may also occur through venereal and congenital infection. Infected mothers who are breastfeeding may transmit the infection to

their infants, the transmission may also occur through tissue transplantation or blood transfusions. Brucellosis is one of the most easily acquired laboratory infections, and strict safety precautions should be observed when handling cultures and heavily infected samples. Person-to-person spread of brucellosis is extremely rare (20-22).

Milk Ring Test was first qualified by Fleischhauer in German in 1937, it is the first line of day to day screening test for individual dairy animal and potentially infected herds for Brucellosis. MRT is a simple, inexpensive, easy, effective method, satisfactory, and takes low time to perform, and is usually the method of choice for the monitoring of dairy herds, it mainly detects IgA and IgM antibodies against *Brucella* infection in raw milk. The sensitivity and specificity of MRT were 85% and 95% consecutively (23, 24).

From a study at hand, the overall occurrence of Brucella antibodies in ewes and nanny goats milk samples was 34/350 (9.7 %). The highest rate of incidence of Brucella antibodies was found in nanny goats milk samples 19/165 (11.5 %), while the lowest rate of incidence was from the ewe milk samples 15/185 (8.1 %) (Table 1). The obtained results indicated that there was a significant difference at the level of 0.05 for the prevalence of Brucella antibodies among ewes and nanny goats milk according to MRT, where the value of Chi-Square was (**227.21**) with the level of significance 0.000 ($p < 0.05$).Our result was approach with percentage found by Ali et al. (25) in Pakistan, who found that among 212 sheep and goats milk samples, only 20 milk samples (9.4 %) were determined as positive by MRT. Also, (26) in Iraq / Al-Samawa city, reported that the prevalence of Brucella antibodies in goats milk samples was 11 / 120 (9.16%) according to MRT, while 5/120 (4.16%) samples were positive according to PCR technique.

In another hand, our results showed a less rate compared with the study conducted by Ibrahim et al.(27) in Egypt whom found that the prevalence rates of Brucellosis using MRT in cattle, buffaloes, sheep, goats, and camels were 51.0, 49.8, 56.2, 36.4 and 34.4%, respectively, with an overall incidence of 47.8% . Abdul-Razag, (28) in Yemen mentioned that the prevalence of Brucella antibodies among raw sheep and goats milk was 17.7% and 14.8% consecutively, and it was evident that all samples which were positive to culture were positive also to the MRT. Dubey *et al*. (29) in India reported that the prevalence of *Brucella antibodies* in milk was 23/85 (27.05 %)

according to MRT, while In PCR, Out of 168 samples 14 samples (8.3%) were found positive. Also, our result incompatible with the result achieved by Khan et al. (30) in Pakistan, who confirmed that the prevalence of *Brucella antibodies* in Buffaloes, Goats and Bulk Tank Milk were 18 out of 300 samples (6.0%) found positive through MRT.

However, lactating females play an essential responsibility in the epidemiology of human Brucellosis, because the *Brucella species* concentrate in the supra mammary lymph nodes and mammary glands in more than 80% of infected females, which persist to excrete Brucella in their milk during its lives, and this is key in its transmission , also in dairies, milking is another mode of transmission if the same teat cups are used for milking, as well as cross contamination that must be taken into account, so these bacteria can be transmitted to consumers through milk and dairies product which represents an essential cause of health risk to society (31, 32).

Simultaneously, milk is a representative medium for monitoring Brucella antibodies, because it is ready, inexpensive and directly obtained, also MRT can be achieved periodically several times, in addition to this test give an excellent expression of blood serological tests, for this reasons MRT persist the mainly practical technique to screen milking females and validate diagnosis of Brucellosis (33).

Result illustrated in Table 2 show that *Brucella isolates* were found in 29 (8.3%) among 350 samples. This result indicated that the isolation of *Brucella species* was high in nanny goats milk 17/165 (10.3 %) compared with samples from ewe milk 12/185 (6.5 %). There is a significant difference at the level of 0.05 for the isolation of Brucella species from ewes and nanny goats milk in Erbil City, where the value of Chi-Square was (243.61) with the level of significance 0.000 ($p< 0.05$).

In the study conducted by Ibrahim et al. **(27)** in Egypt, they examined 881 milk samples by different assays showed an incidence of 47.8% by MRT, 42.8% by ELISA; 33.8% by isolation, 50.2% by PCR assays and 45.3% by DBH assays**.** Gulbaz and Kamber (34) in Turkey, mentioned that from 215 raw milk samples only 4 (1.86%) samples were Brucella positive.

Ashrafganjooyi et al. (35) in Iran confirmed that nine milk samples out of 700 (1.28%) were positive by the bacteriological method and all of them were *Brucella melitensis* Biotype 1 and one out of 700 samples was *Brucella ovis.*

Shirazi **et al. (36)** detected *Brucella* species in 10 milk samples including two samples from apparently healthy animals (1 sheep sample, and 1 goat sample) as well as eight samples from animals with abortion history (6 sheep samples, and 2 goat samples) by using culture method.

In another hand the result achieved by Moslemi et al., (12) in Iran, illustrated that the prevalence of *Brucella* species contamination in the dairy products was: 45.5% in goat's raw milk, 39.1% in non-pasteurized cheese, 27.3% in sheep's raw milk, 26.3% in cow's raw milk, 25% in pasteurized cheese, and 14.7% in pasteurized milk.

Anyway after ewe and nanny goats are infected with *Brucella species*, during lactating period their milk is contaminated with this type of bacteria, and milk-producing animals remain carriers and shed the *Brucella* in their milk for prolonged durations, in addition, if the milk is not pasteurized, these bacteria can be transmitted to people who drink milk or consume dairy products prepared from it (37).

When we study the correlation between the result of MRT and isolation of *Brucella species* from ewe and nanny goats milk , we noticed that 15 / 185 (8.1 %) and 19 / 165 (11.5 %) samples from ewes and nanny goats were positive according to MRT, compared with 12/185 (6.5 %) and 17/165 (10.3 %) samples gave isolates of Brucella species respectively (Table 3).

The sensitivity of MRT were 80.0 % and 89.5 % in case of milk samples collected from ewe and nanny goats consecutively, while the specificity of MRT were 93.5 % and 89.7 % for milk samples collected from ewe and nanny goats respectively. Ibrahim et al. (27), reported that the sensitivities of MRT were 100%, 100, 93.1, 93.02 and 84.21% in case of milk samples collected from cows, buffaloes, sheep, goats, and camels, respectively. Simultaneously, specificities of MRT were 75.12%, 73.51%, 72.72%, 98.71% and 86.66% for milk samples collected from cows, buffaloes, sheep, goats, and camels, respectively.

According to the Table 4 , we noticed that 5 / 12 (41.7%) and 3 / 17 (17.6 %) of isolates were *Brucella abortus* , while 7 / 12 (58.3%) and 14 / 17 (82.4%) were *Brucella melitensis* consecutively . These observations indicate that *Brucella melitensis* was the predominant species in ewe and nanny goats milk. There is no significant difference found between the prevalence of *Brucella species Br. abortus* and *Br. melitensis*) in ewes and nanny goats milk (P >0.05).

Our result was inconsistent with research of Shakerian et al. (38) in Iran, who

revealed that out of 125 sheep milk, 12 (9.6 %) had *Brucella melitensis*, and out of 100 goat milk samples, 18 (18 %) were positive for *Brucella melitensis*. These findings show that *Brucella melitensis* is present in a significant proportion of caprine and ovine milk in a section of Iran.

Another point of this work includes the relation between months and results of MRT through a period of study were followed up. Data which illustrated in Table 5, explain that the prevalence increased on April 10 /65 (15.4%). then on March 6 / 55 (10.9%), while the lowest rate was found in May and June 5/ 65 (7.7 %) for each month. The value of (P<0.05)(0.00) showed a significant difference for the relation between months and prevalence of Brucella antibodies among ewes and nanny goats milk according to MRT during the period of study.

The results at hand in agreement with the study carried out by 39 in Saudi Arabia, who reported that the highest number of cases was in April to June ($n = 361$; 39.5%) and the lowest cases were reported in January. Also, 40 in the Kingdom of Saudi Arabia analysis the tendencies of recorded human cases of brucellosis during 2004– 2012, confirmed that most cases were reported among spring and summer seasons. In support of our result, (41) in Kenya declared that the highest cases of brucellosis occurred through July, then September, March, and October. They detected that the most of the high occurrence of brucellosis happen during the rainy season, while the lowest cases were noticed in December, then May and January.

Nematollahi et al. (42) in Hamadan Province, Iran, observed that the highest relative occurrence of new cases was monitored in the summer season 94.5 %, while the relative incidence of recurrent cases was 5.49 % in summer, 6.18 % in autumn, 8.55 % in winter and 6 .21 % in spring. Also, (43) in China, revealed that 99.3% from a total of 513,034 human brucellosis cases were reported in northern China among 1955–2014, and 69.1% (258, 462/374, 141) take place throughout February–July in 1990–2014.

In Iran (44), explained that the mainly recorded cases were in June and July and the lowest statistic occurred in January. It shows that the interval of the disease initiate in the spring and reaches its peak in the summer, and then begins to decline in autumn.

From this investigation, we concluded that MRT plays a significant role in the revealing of Brucellosis in milk. The general prevalence of Brucella antibodies among ewes and nanny goats milk in Erbil Governorate seems

to be high (9.7 %), and this proportion regard as hazardous to public health.

Due to the importance of this work, we recommend that the consumers should remember that milk necessarily to be perfectly heated to destroy this foodborne pathogen, and propagation of health awareness through the media (audio, visual media and newspapers), highlighting the method of transmission of *Brucella species*. This research also underlines for putting in the ground MRT in the diagnosis of this bacteria mainly in collection centers of milk, in dairy factories, and in the field by veterinarians to eradication and control of brucellosis in dairy cows.

CONCLUSIONS

Brucellosis is a great health concern and economically significant in different areas including the Kurdistan region. The genus Brucella is comprised mostly of mammalian pathogens, and the key species are the milk-producing animals, so milk and dairy products play a significant role in the transmission of Brucella to human, and the hazards increased due to their low infectious dose of 10–100 organisms is sufficient to cause systemic infection. We concluded that MRT can be used for fast routine monitoring of lactating ewes and nanny goats because this test is a simple procedure for day to day screening of Brucellosis in milk. The findings of this work show that a sizeable percentage of prevalence of Brucella antibodies among ewes and nanny goats milk in Erbil Governorate seems to be high (8.1 %) and (11.5 %) consecutively, and this percentage in ovine and caprine milk consider dangerous on public health.

Due to the consequence of this work, we recommend that the consumers, should remember that milk needs to be properly heated to destroy this milk-borne Bacteria, and broadcasting of health knowledge through the media (audio, visual media and newspapers), highlighting the method of transmission. This study also underlined for using MRT in the diagnosis of Brucella antibodies in different steps of milk production to eradication and control of brucellosis in milk-producing animals.

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