Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 50, Number 3, p. 228-240, September 2022
DOI: 10.21608/zvjz.2022.145521.1184

RESEARCH ARTICLE

Prevalence and Molecular Differentiation of Brucella Species in Buffaloe Raw Milk and Karish Cheese in Egypt

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Article History: Received: 22/06/2022 Received in revised form: 25/07/2022 Accepted: 2/09/2022

Abstract

Ninety buffalo's milk samples included 20 bulk tank milk samples from small dairy farms and 70 from retail milk at different localities in Dakahlia Governorate. Forty-eight individual milk specimens were obtained from animals positive for Rose Bengal Test (RBT), buffered acidified plate antigen test (BAPAT) together. Milk samples were tested with a milk ring test (MRT), followed by isolation and identification of the prevalent serotypes. At the same time, conventional PCR was applied. One hundred and sixty seven buffalo's serum samples were collected from 12 smallholder's herds and their bulk tanks gave positive to MRT were confirmed by BAPAT and RBT. A total of 50 karish cheese were collected from street vendors and supermarkets located in Mansoura city, Dakahlia Governorate, Egypt. Conventional PCR was also used to detect Brucella DNA at the species level on the collected cheese samples. The results showed that the prevalence of brucellosis in small dairy buffalo farms was 60% by MRT and 28.7% though using RBT and BAPAT. The prevalence of *Brucella* in buffalo's retail milk was 20% in each of Bilqase, Sherbin, and Talkha, 30% in each of Aga and Nabroh, 10% in Sinblaween. However no positive results was revealed in Mansoura. The isolation rate of *Brucella* was 31.25% in individual positive samples and 7.69% in retail positive milk samples. While using conventional PCR, the percentage reached 62.5% in individual positive samples and 69.23% in retail positive milk samples. All *Brucella* isolates (n = 16) were biochemically identified into three *Brucella abortus biovar1* (18.75%) and 13 *Brucella melitensis biovar 3* (81.25%). The prevalence of *Brucella* in examined Karish cheese was 20%. All of the tested isolates were verified using conventional PCR. Raw milk and unpasteurized soft cheese have public health risks for humans and are possible sources of transmitting *Brucella* and must be under regular and mandatory food control measures.

Keywords: *Brucella*, Buffalo, Raw milk, Karish cheese.

Introduction

Brucella since was discovered in 1939 in Egypt, despite the efforts made by the veterinary authorities to control the spreading of infection among different domestic animals, *Brucella* is considered the most important zoonotic disease after rabies [1].

Egyptian population has dramatically increased year by year and the expected growth is 65% in 2050. This horrific population increase will be accompanied by an increase not only in meat, egg, and good quality food consumption but also in milk and milk by-products by over 300% [4]. Utilization of milk per person in Egypt increased from 77.9 kg in 2012 to nearly 91.7 kg in 2020 with a raising usage rate of around 1.8%. This increase in dairy milk and its by-product demand due to the successive population increase, especially in the number of children, in addition to raising awareness of

health and the increase in the standard living of the individual[5,6]. At the same pace, Egyptian smallholders are responsible for more than 70% of total milk production. Cows are milked via traditional methods, a poor hygienic conditions during transportation and collection of milk. Moreover, this point becomes more dangerous because the vast majority of that milk is sold directly to family usage or for marketing in mini market [7, 8]. So, more attention should be applied to researchers and veterinary authorities to increase population awareness about *Brucella* transmission via raw milk, and unpasteurized dairy products like Karish cheese which consider hazardous factors that lead to acquiring disease from eating [9].

Karish cheese is low-salt soft cheese utilized in Egypt due to its relatively low price and high nutritive value, especially in the countryside. Various types of bacteria can contaminate cheese during manufacture, processing, transportation, and keeping in unsanitary settings [10]. *B. melitensis* can survive in Karish cheese manufactured from naturally contaminated unpasteurized milk, with a survival rate of up to the eighth day at ambient temperature, while it can survive in contaminated unpasteurized milk for up to five days when kept at 4°C and up to nine days at -20°C [11].

The incidence of *Brucella* in Egypt differs among researchers due to several issues, for example, the number of investigated animals, the season of work, and the used serological tests. Moreover, they concluded that definite detection of positive cases requires using more than one serological test [12]. Serological diagnosis of *Brucella* on serum samples was performed through Rose Bengal Test (RBT) and Buffered Acidified Plate Antigen Test (BAPAT), while milk ring test (MRT) in milk is still our first choice to catch the positive cases. MRT, RBT, and BAPAT are the most prevalent screening serological tests but eradication programs can't depend on MRT alone because little titer of antibodies in milk and factors of fat collecting lead to decrease its sensitivity [13, 14]. MRT not only be used in bulk milk samples analysis but used also to test the individual milk samples. However, it should be confirmed by other serological tests because it may give false positive results as in cases of mastitis, at the late stage of pregnancy or immediately after parturition [15].

Brucella can be a persistent infection in the udder with intermittent descending in the milk of the infected animals, so isolation from milk is hard work [16]. Two strains of *Brucella* are the common isolates in Egypt; *B. abortus* and *B. melitensis*, on the same time *B. melitensis* biovar 3 is a famous strain-affected animal in Egypt [17]. Detecting organisms using polymerase chain reaction (PCR) has been proven to be more efficient than culture isolation. Although PCR can be used to diagnose *Brucella*, only a few research have been conducted using field samples to see if it can be used as a diagnostic tool [18]. The sensitivity of genus-specific PCR proved to be higher than cultivation because it can detect even low concentrations of DNA in the samples [19]. The purpose of this investigation was to determine the prevalence of *Brucella* in raw buffalo milk and Karish cheese samples in Dakahlia Governorate, Egypt.

Materials and Methods

Samples

*Milk samples***:**

A total of 90 buffalo's milk samples were collected from bulk tanks (20 bulk tank samples from small dairy farms owned by smallholders (1:20 buffalo) and 70 bulk tank samples from retail milk at different localities in Dakahlia Governorate) to investigate the prevalence of *Brucella* during the period between January 2016 and December 2017. Forty-eight individual milk samples were collected from the serologically positive animals to RBT and BAPAT.

Positive milk samples to MRT were cultured for isolation and identification of the prevalent serotypes. At the same time, conventional-PCR was applied on the positive milk samples and the obtained isolates by culture methods.

*Serum samples***:**

A total of 167 buffalo serum samples were collected from 12 smallholder's farms whose bulk tank gave positive to MRT.

*Soft cheese***:**

A total of 50 karish cheese samples were collected from street vendors and supermarkets located in Mansoura city, Dakahlia Governorate, Egypt to prove its public health hazard *Brucella*. Conventional PCR was also used to detect *Brucella* DNA at the species level on the collected cheese samples.

Milk samples were investigated by MRT and serum samples were serologically analyzed using RBT and BAPAT according to Alton, and Jones Animal Health and Veterinary Laboratories Agency provided the antigen (AHVLA), New Haw, Addlestone, Surrey KT15 3NB, UK [15]. The Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt, provided antigens and test materials.

Brucella isolation

Bacterial isolation was carried out on the positive milk to MRT from the serologically positive individual cases (48 milk samples) and the positive samples from the retail milk (13 milk samples). Utilizing Bacto-*Brucella* agar (Difco Laboratories, Detroit, Mich, USA) with and without antibiotics (Cyclohexamide (actidione, 50mg), Bacitracin (12.500 units), Polymyxin B sulfate (2500 units), Vancomycin (10mg), Nalidixic acid (2.5 mg) and Nystatin (50000 units) [20]**.** The culture operations were carried out in a biohazard safety cabinet with a class 2 type A/B3 classification (model no. NU-425-400E, Nuaire TM, Plymouth, MN 55447, USA). The cultured plates were incubated aerobically at 37°C in a CO_2 incubator with 10% CO2 (model no. 322-11, NAPCO®, National Appliance Co., Oregon, USA). After five to seven days, cultivated plates were checked for any growth up to 35 days. To obtain a sediment-cream mixture, milk samples were centrifuged at 3000 rpm for at least 10 minutes. Bacterial isolation was carried out at Animal Health Research Institute- Dokki-Egypt, according to the FAO/WHO Expert Committee's recommendations on *Brucella* [15, 21].

Bacterial identification

All isolates were identified by a scheme of biotyping analysis according to Alton et al. [15] and OIE [21].

Molecular typing

Brucella isolates were again categorized molecularly at the species level. Conventional PCR was carried out as previously described by Sambrook, Fritscgh [22] and Bricker and Halling [23]. Oligonucleotide primers used in cPCR as mentioned in Table (1). Briefly, extraction of DNA was applied according to QIAamp DNA mini kit (Catalogue no.51304) instructions. 20 µL QIAGEN protease was added to 200 µL of the sample plus to 200 µL buffer AL, mixed for 15 sec, then incubated at 56˚C/ 10 min followed by centrifugation. Then 200 µl ethanol (96%) was added to the sample and mixed for 15 sec. The mixture was carefully applied to the QIAamp Mini spin column and centrifuged at 8000 rpm for 1 min. Then 500 mL buffer AW1 was added and centrifuged at 8000 rpm for 1 min. The QIAamp mini spin column was placed in a clean 2 mL collection tube then 500 ml buffer AW2 was added and centrifuged at full speed for 3 min. Centrifugation at full speed for 1 min was done. The QIAamp mini spin column was carefully opened and 100 µL buffer AE was added and incubated at room temperature (15-25˚C) for 1 min, and then centrifuged at 8000 rpm for 1 min. PCR amplification was applied as following: primary denaturation at 94°C for 5 minutes then 35 cycles of secondary denaturation (94°for 30 seconds) followed by annealing at 55°C for 1 minute, and extraction at 72°C for 2 minutes followed by final extraction at 72°C for 10 minutes. 6 µL of the ladder was mixed gently followed by gel electrophoreses. Gels were stained with ethidium bromide and photographed by a gel documentation system and the data was analyzed through computer software. Visible

bands of appropriate sizes of 498 bp for *B. abortus* and 731 bp for *B. melitensis* were regarded as positive reactions.

Table 1. Oligonucleotide primer sequences used in the study (Metabion, Germany)

Target gene	Target	Primers sequences (5′to3′)	Amplified segment (bp)	Reference
1S711	*B. abortus*	F: TGCCGATCACTTAAGGGCCTTCAT R: GACGAACGGAATTTTTCCAATCCC	498	**[23]**
	B. melitensis	F: TGCCGATCACTTAAGGGCCTTCAT R: AAATCGCGTCCTTGCTGGTCTGA	731	

Results

Prevalence of Brucella in small dairy buffalo farms

To establish the prevalence of *Brucella*, MRT was used to test 20 bulk tank samples from small buffalo dairy farms for *Brucella* antibodies, followed by the collection of serum samples from positive bulk tank samples. 167 buffalo serum samples were taken from 12 smallholder farms, and their bulk tanks tested positive for MRT and were investigated by RBT and BAPAT. MRT discovered that 60% (12 milk bulk tank samples) were positive. An investigation of 20 bulk tank milk samples (225 animals) by RBT and BAPAT found that 28.7% (48 animals) were positive in the current study (Table 2).

Prevalence of Brucella in buffalo's retail milk at different localities:

Table (3) displays the results of using MRT to determine the prevalence of *Brucella* of a total of 70 bulk tank samples from retail milk collected from different localities in Dakahlia Governorate. The prevalence of *Brucella* in buffalo's retail milk at different localities was 20% in Bilqase, Sherbin, and Talkha, 30% in Aga and Nabroh, 10% in Sinblaween and 0.0% in Mansoura.

Comparison between the sensitivity of the bacteriological method and Conventional-PCR in the detection of Brucella from milk

Table (4) demonstrates the results of bacteriological investigation of 48 individual positive milk samples from small farms and 13 retail positive milk samples, as well as identification of 16 *Brucella* isolates, in comparison to conventional PCR testing. The isolation rate from individual positive milk samples (48 samples) was 31.25%, but the rate using conventional PCR was 62.5%. Meanwhile, the isolation rate in retail positive milk samples (13 samples) was 7.69%, compared to 69.23% by conventional PCR (Figures 1-4). Three of the 16 *Brucella* isolates were *B. abortus biovar 1* (18.75%) and 13 (81.25%) were *B. melitensis* biovar 3.

Result of examining soft cheese by using conventional PCR

The results of examination of a total of 50 karish pieces of cheese were collected from street vendors and supermarkets using conventional–PCR showed that the prevalence of *B. melitensis* was 20%. Due to the precision and speed of this procedure, karish cheese samples were tested immediately utilizing conventional–PCR without bacteriological isolation (Table 5).

Table 2. Prevalence of *Brucella* in small dairy buffalo farms.

Farm	Animal No.	MRT +ve *	No. positive animal **
Farm 1	10	+	3
Farm 2	8	+	2
Farm 3	5	-	0
Farm 4	15	+	6
Farm 5	20	+	8
Farm 6	18	+	4
Farm 7	10	-	0
Farm 8	13	-	0
Farm 9	3	-	0
Farm 10	5	-	0
Farm 11	10	+	2
Farm 12	8	-	0
Farm 13	11	+	2
Farm 14	20	+	6
Farm 15	6	-	0
Farm 16	14	+	3
Farm 17	20	+	5
Farm 18	8	-	0
Farm 19	11	+	2
Farm 20	10	+	5
Total (20 farms)	225	12 (60%)	48 (28.7%)

*Positive milk bulk tank for MRT.

**Positive for the serological test (RBT and BAPAT).

Table 3. Prevalence of *Brucella* in buffalo's retail milk at different localities.

Town	Samples No.	Positive samples (MRT)	%
Bilqase	10	2	20%
Aga	10	3	30%
Sherbin	10	2	20%
Nabroh	10	3	30%
Sinblaween	10	1	10%
Talkha	10	2	20%
Mansoura	10	0	0.0%
Total	70	13	18.5%

Table 4. Comparison between the sensitivity of the bacteriological method and conventional PCR in the detection of *Brucella* from milk.

Method of *Brucellae* detection	Individual positive milk samples (48 samples)		Retail positive milk samples (13 samples)		Total +ve	Identification of (16) isolates	
	+ve	-ve	+ve	-ve		*B. abortus biovar 1*	*B. melitensis biovar 3*
Bacteriological method	15 (31.25%)	33 (68.75%)	1 (7.69%)	12 (92.30%)	16 (26.22%)	3 (18.75%)	13 (81.25%)
Conventional PCR	30 (62.5%)	18 (37.5%)	9 (69.23%)	4 (30.76%)	39 (63.93%)		

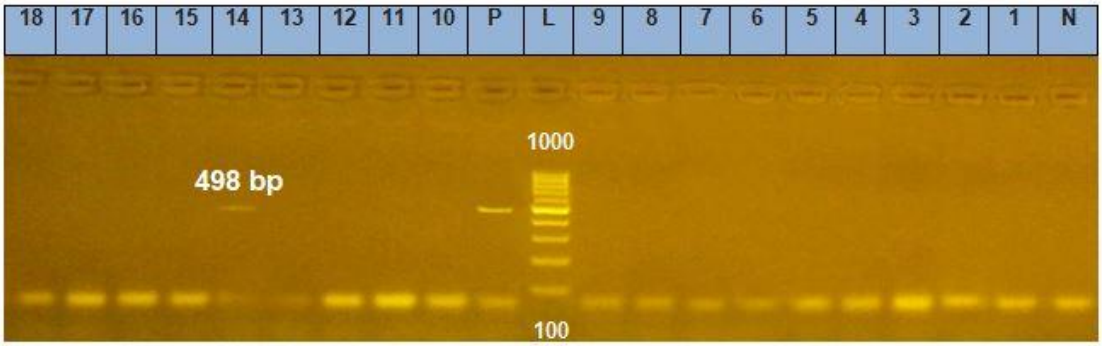

Figure 1: Agarose gel electrophoresis of amplified PCR products from *Brucella* isolates DNA. Lane (14) showed positive results for the presence of the *B. abortus IS711* gene (428 bp). L: Ladder (size range 100-1000 bp),bp: base pair, P: positive control, N: Negative control.

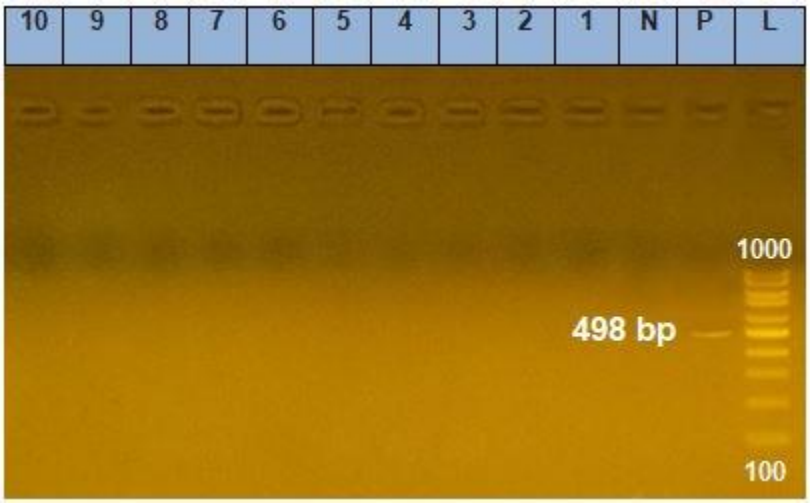

Figure 2: Agarose gel electrophoresis of amplified PCR products from *Brucella* isolates DNA. Lanes (1- 10) showed negative results for the presence of the *B. abortus IS711* gene (428 bp). L: Ladder (size range 100-1000 bp),bp: base pair, P: positive control, N: Negative control.

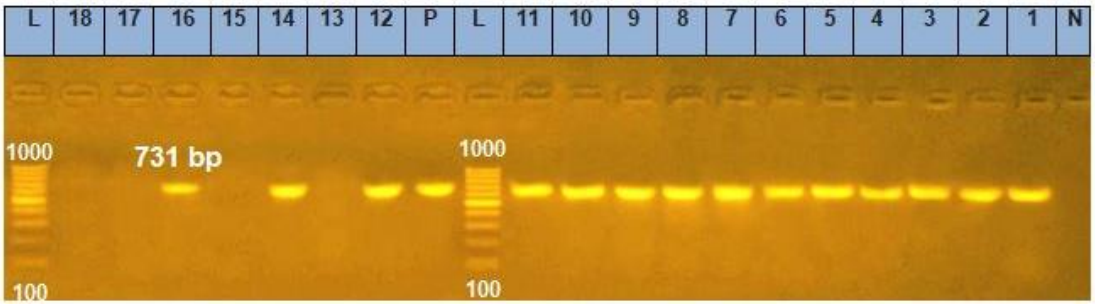

Figure 3: Agarose gel electrophoresis of amplified PCR products from *Brucella* isolates DNA. Lanes (1-12 and 14) showed positive results for the presence of *B. melitensis IS711* gene (731bp). L: Ladder (size range 100-1000 bp), bp: base pair P: positive control, N: Negative control.

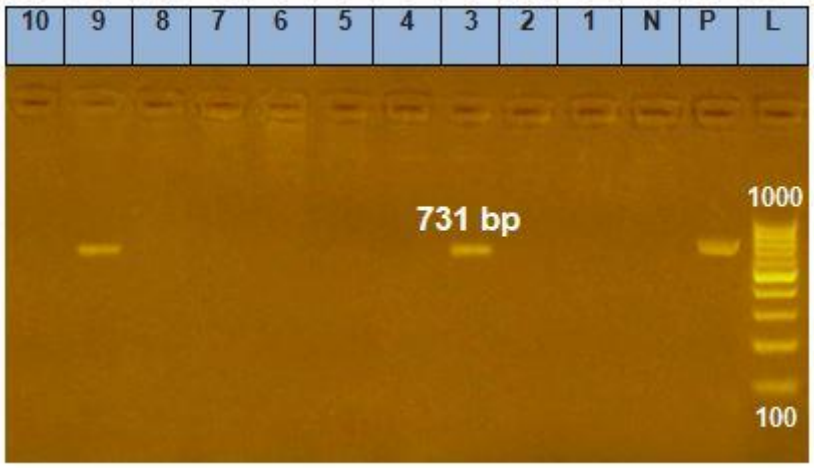

Figure 4: Agarose gel electrophoresis of amplified PCR products from *Brucella* isolates DNA. Lanes (3 and 9) showed positive results for the presence of the *B. melitensis IS711* gene (731bp). L: Ladder (size range 100-1000 bp), bp: base pair P: positive control, N: Negative control.

Table 5. Prevalence of *Brucella* in soft cheese.

***Brucella* detection by conventional PCR**	**Soft cheese samples (50 samples)**		**Total +ve**
	Positive	**Negative**	
B. melitensis	10	40	10
B. abortus	0	50	

Discussion

Brucella induces systemic disease and can be identified in mammary gland tissue and related lymph nodes, as well as being shed in vast numbers in the milk and is considered one of the most hazardous health problems, particularly in developing nations [24].

A milk ring test (MRT) was applied on 20 bulk milk tank samples collected from smallholder farms, followed by the collection of serum samples from individual positive animals. The milk ring test is a quick and inexpensive way to diagnose *Brucella* in dairy cows [15]. Furthermore, the viability of MRT was shown to be higher than that of other serological assays [25]. After evaluating 20 milk bulk tank samples, MRT discovered that 60% (12 milk bulk tank samples) were positive (Table 2). Meanwhile, 70 retail milk samples were collected from diverse places and examined using MRT. The prevalence of *Brucella* in buffalo's retail milk at different localities was 20% in Bilqase, Sherbin, and Talkha, 30% in Aga and Nabroh, 10% in Sinblaween and 0.0% in Mansoura. The overall prevalence was 18.5% (Table 3).

Our results were higher than a previous study of Abbas and Aldeewan [26] in which the prevalence rates of brucellosis by using MRT was 35% in milk buffalo samples. Cadmus, Adesokan [13] found that prevalence of *Brucella* in milk samples by MRT was 18.61%.

In an endemic region, using many serological tests for screening affected cases is essential for eradicating the disease within a herd. Also, releasing the herd from quarantine too soon should be avoided, especially in unsanitary settings and with uncontrolled animal movement. Although, the herd should be examined by a series of serological tests to identify the animals that may still be infected with the disease. Veterinary authorities' standards allow quarantined herds to be released after three consecutive negative serological examinations, as is well known [27]. Antibodies to *Brucella* species are routinely detected using agglutination assays

such as BAPAT and RBPT [28]. An investigation of 20 bulk tank milk samples (225 animals) found that 28.7% (48 animals) were positive in the current study (Table 2).

Also Hassan [11] reported that the overall prevalence was recorded as 52% in serum samples by BAPAT. The current results near to those obtained by El-Sayed, El-Newishy [29] who found the prevalence of brucellosis by using MRT was 62.1% and 53.2% in cows and buffaloes milk samples. While Hossam Eldin and coauthors [30] screened serum samples by BAPAT and 25% from samples which serologically positive to *Brucella*.

Isolation and identification of *Brucella* is still the main way for certain detection and it's a crucial tool for confirming the serological results. In this investigation, the isolation rate from individual positive milk samples (48 samples) was 31.25%, but the rate using conventional PCR was 62.5%. Meanwhile, the isolation rate in retail positive milk samples (13 samples) was 7.69%, compared to 69.23% by conventional PCR (Table 4).

Brucella spp. were isolated from milk samples in the current investigation, which is problematic because *Brucella* organisms colonized the supramammary lymph nodes and mammary glands in 80% of infected dairy animals, resulting in the infection being excreted in milk throughout the animal's life [31] . *Brucella* organisms can also survive for four days in raw milk or water, and when the number of organisms is low, *Brucella* multiplies by five log units in three weeks, providing a risk to human consumers [32].

Conventional-PCR has a higher sensitivity and specificity for *Brucella* detection than the culture approach according to our findings. When compared to bacteriological culture methods, the high positive milk samples by conventional PCR might be due to the very low quantities of bacteria, which would be compatible with the minimal number of colony-forming units discovered in milk samples by culture methods [18], and because PCR can identify the two die and live bacteria, whereas traditional culture method can detect live creatures only [33]. Furthermore, the culture process takes time and poses a significant risk of infection to laboratory employees [34].

The demand for additional ambient 10% CO_2, formation of hydrogen sulfide gas, development of urease, growth in medium containing the inhibitory dyes thionin and fuchsin, and agglutination with polyclonal monospecific antisera A, M, and R were all employed to delineate biovars. Most isolates of *B. melitensis* were typed as biovar 3 according to the results. According to previous records, this biovar was identified and regarded as the most common kind in Egypt [2, 35, 37].

As exposed in Table (4), three of the 16 *Brucella* isolates were *B.abortus biovar 1* (18.75%) and 13 (81.25%) were *B. melitensis* biovar 3. This is because Egypt has mixed populations of sheep, goats, cattle, and buffaloes that are kept together, and *B. melitensis* was the most widespread strain among cattle and the causative agent of *Brucella* [2, 38-41] .

According to previous studies, Abbas and Aldeewan [26] found 19 isolates in Buffaloe's milk were *B. abortus* and *B. melitensis* with percentages of 73.6% and 26.4%, respectively**.**

Karish cheese is a soft acid cheese that is widely produced in Egypt. It is a soft, white curd, acid-coagulated fresh cheese prepared from skimmed milk (cow milk, buffalo milk, or buttermilk from sour cream) [42]. When compared to culture-based methods, real-time PCR for pathogen detection in food have accuracy and sensitivity. Furthermore, the used PCR technique is unable to establish the existence of living bacteria; as a result, the findings may be significantly under - or overestimated. The preliminary level of contamination in the milk, the kind of thermal treatment, homogenization, and fat-standardization of the milk, a ripening process and storage conditions (humidity, temperature), the pH value as well as the salt content of the cheese, and also the duration between production and testing, all play a role

in *Brucella* spp. isolation from cheese [43-45].

The results in Table 5 presented that the prevalence of *Brucella* was 20% in the tested Karish cheese samples. All of the isolates were *B. melitensis* by using conventional PCR. While Jansen et al. [46] explained that positive *Brucella* cheese samples were divided into 4 and 37 samples prepared from pasteurized milk, and raw milk cheese, according to the vendor's information. Only three of the 41 positive cheese samples tested positive for alkaline phosphatase, requiring the categorization of 38 samples as pasteurized cheese. The prevalence of *Brucella* DNA in raw milk cheese samples was not significantly higher than in pasteurized milk cheese samples (P= 0.08), and there was no difference across the cheeses (P= 0.59). *Brucella* DNA was discovered in cheese produced in European Union Member States (EU MS, 23%) and non-EU MS (22%).

Kara and Akkaya [47] found *Brucella* in fresh cheese samples (2% *B. abortus* and 7% *B. melitensis*). Abbas and Talei [48] found 8 *Brucella* isolates from cheese samples. They were 5 *B. melitensis* and 3 *B. abortus*. While Pamuk and GÜRler [49] isolated 28 isolates which were 18 (9%) *B. abortus* and 10 (5%) *B. melitensis*.

In Egypt, raw milk used in the production of karish cheese was linked to 62.1% of *Brucella* cases [9]. Other Egyptian studies revealed the same results [50, 51].

Although Karish cheese is a good source of nutrients, there is a risk of developing *Brucella* if humans consume non-pasteurized dairy products that have not been exposed to standard sanitary and health procedures [52]. Meanwhile, *Brucella* strain survival in various dairy products is inversely proportional to pH [53, 54]. *B. melitensis* and *B. abortus* were isolated in cheese manufactured with raw milk and ripened at 24 ˚C at pH 4 and aw 0.89 in a very high count [55, 56]. Almost all previous studies on the influence of pH on *Brucella* organisms concluded that product pH plays a critical role in *Brucella* spp. survival and proliferation in dairy products. The authors proposed a direct relationship between microbe survival and pH, as well as the possibility of predicting the role of dairy products as a vehicle for spreading numerous infections by knowing the pH values of the products [44, 53, 57, 58]. The survival of *Brucella* in dairy products is known to be influenced by pH and water activity.

At 37°C, the ideal pH for *Brucella* spp. survival and growth are between 6.6 and 7.4 [59].

Conclusion

Routine screening of animals for brucellosis is crucial, that may help to detect positive cases and reduce the risk of disease transmission. A combination of serological tests such as BAPAT and RBPT, followed by bacterial isolation and identification by PCR can be used for accurate diagnosis of *Brucella*. Effective implementation of control measures including test and culling of infected animals, and quarantine and movement controls may prevent the spread of infection. Application of hygienic measures could help in control of brucellosis in the dairy farms. Also pasteurization of milk and dairy products can prevent the transmission of zoonotic disease as brucellosis.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

The authors would like to acknowledge the supports from the Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.

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الملخص العربي

معدل انتشاروالتصنيف الجزيئى لميكروب البروسيلا فى حليب الجاموس الخام والجبن القريش فى مصر

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أجريت الدراسة على إجمالي 90 عينة من حليب الجاموس مقسمه إلى 20 عينة حليب مجمعة من مزارع الألبان الصغيرة و 70 عينة حليب مجمعة من حليب التجزئة في مواقع مختلفة في محافظة الدقهلية. تم جمع عدد 48 عينة حليب فردية من الحيوانات الموجبة المصل لـ RBT و BAPAT. تم اختبار عينات اللبن باختبار اللبن الحلقى (MRT). ثم بعد ذلك تم عزل وتصنيف الأنماط المصلية السائدة في نفس المكان.بالاضافة إلى تطبيق تفاعل البلمرة المتسلسل (PCR) على المعزولات التي تم الحصول عليها بواسطة طرق الاستزراع. تم جمع عدد 167 عينة من مصل الجاموس من 12 مزرعة لأصحاب الحيازات الصغيرة والتي أعطت خزان السائب إيجابيًا لـ MRT ولمزيد من التأكيد كان من خلال BAPAT و RBT. تم جمع عدد 50 جبنة قريش من الباعة الجائلين والمتاجر الكبيرة الموجودة في مدينة المنصورة ، محافظة الدقهلية ، مصر. تم أستخدام تفاعل البلمرة المتسلسل (PCR) أيضًا للكشف عن DNA الخاص بـالبروسيلا على مستوى الأنواع في عينات الجبن التي تم جمعها. أظهرت النتائج انتشار ميكروب البروسيلا في مزارع جواميس الألبان الصغيرة بنسب 60٪ و 28,7٪ بواسطة MRT و (RBT و BAPAT) على التوالي. كانت نسبة انتشار ميكروب البروسيلا في ألبان الجاموس بالتجزئة في مواقع مختلفة 20٪ ، 30٪ ، 10٪ و 0,0٪ في (بلقاس ، شربين ، طلخا) ، (في أجا ونبروه) ، (السنبلاوين) و (المنصورة) على التوالي. بلغ معدل الانتشار الكلي 18.5٪ ، وتم العزل بنسب 15 (31,25٪) و 1 (7,69٪). بينما باستخدام PCR كانت المعدل 30 (62,5٪) و 9 (69,23٪) في عينات اللبن الموجب الفردي وعينات الحليب الموجب التجزئة، على التوالي. تم تحديد معزولات ميكروب البروسيلا بيوكيميائيا على أن 3 (18,75٪) و 13 (81,25٪) 1 biovar *B.abortus* و *B.melitensis* biovar 3. كانت نسبة انتشار البروسيلا في جبنة قريش المدروسة 20٪. جميع المعزولات كانت موجبة لبكتريا *B.melitensis* والتي تم التحقق منها باستخدام تفاعل البلمره المتسلسل .واثبتت الدراسة أن الحليب الخام والجبن الطري غير المبستر لهما مخاطر صحية عامة على الإنسان ومصدر جيد لنقل ميكروب البروسيلا للإنسان.