Investigation of *Burcella* spp. from Locally Produced Cheeses in Baquba city- Iraq

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Abstract

Brucellosis is considered as one of the most important infectious diseases. This disease caused by different species of *Brucella*. The main source of human brucellosis are dairy products , especially cheese made from unpasteurized raw milk. The aim of this study was to investigate the prevalence of *Brucella spp.* From locally produced cheese "Gibin Al-Arab" made from unpasteurized milk in Baquba city. For this purpose, a total of fifty cheese sample was collected from street shops of the old town in Baquba, from May 2015 till September 2015. Conventional culture method has been used in the isolation. *Brucella* spp. has been detected at the rates of 12% and were distributed in to two species, *B. melitensis* 8%, the rest were identified as *B. abortus* at rate of 4%, Consequently It has been accepted that fresh cheese "Gibin Al-Arab" samples are contaminated with *B. abortus* and *B. melitensis* . This study was conducted at the laboratory of Microbiology, Department of Pathological analysis, Baquba Technical Institute, Middle Technical University. Diyala-Iraq.

Keywords : *Burcella abortus*, *Burcella melitensis*, white cheese, Baquba,

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التحري عن انواع جراثيم البروسيلا المعزولة من الجبن المحلي في مدينة بعقوبة - العراق

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الخلاصة

يعتبر داء البورسيلات (Brucellosis) (حمى مالطا) من الامراض المعدية التي تسببها انواع مختلفة من جراثيم البروسيلا. تعتبرمشتقات الحليب وخصوصا الجبن المصنع من الحليب الخام المصدر الرئيسي للاصابة بهذا المرض في الانسان.كان الهدف من هذا البحث هو التحري عن وجود انواع البروسيلا في الجبن المصنوع محليا من الحليب الخام الغير المبستر (جبن العرب) في مدينة بعقوبة، لهذا الغرض تم جمع (50) عينة من الجبن المحلي من (شهر مايس 2015 الى شهر ايلول 2015) الذي يباع من قبل الباعة المتجولين في اسواق مدينة بعقوبة القديمة. وتم استخدام طرق الزرع المختبرية في عزل وتشخيص الجراثيم. بينت الدراسة ان نسبة تلوث العينات بأنواع البروسيلا كانت (12%). شكلت جراثيم النوع (*B. melitensis*) نسبة 8% والباقي تم تشخيصه على اساس نوع البروسيلا (*B. abortus*) اي بنسبة 4%. بالنتيجة فان تلوث الجبن المحلي المصنوع (جبن العرب) بجراثيم البروسيلا (*B. melitensis*) و(*B. abortus*) تشكل تهديدا على الصحة العامة.

الكلمات المفتاحية : بروسيلا المُجهضة ، بروسيلا المالطية، الجبن الابيض، بعقوبــة

Introduction

White cheese that called locally " Gibin Al-Arab" is one of a traditional fresh soft cheese generally made from unpasteurized milk in different cities of Iraq .The short-ripening time and production from unpasteurized raw milk can facilitate bacterial contamination, reports regarding food-borne disease outbreaks involving dairy products and food surveillance indicate fresh cheese as a good source of food pathogens such as *Brucella spp*.(1) .

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Brucellosis is one of the most important infectious and is a widespread zoonosis disease which transmitted to human either by direct contact with infected animals or by consuming of contaminated animal products, specifically unpasteurized milk and soft cheese (2).

Brucella infection is seen in veterinarians, livestock producers, animal products processing and laboratory workers (3). Fresh white cheese which is produced locally and sometimes in houses from raw sheep and goat milk is may be the most important way of transmission of the disease (4). Sheep, goats and cow were the primary domestic animals in Iraq. Raw milk of small ruminants was used to make cheese. It was therefore hypothesized that milk and milk products were important sources of an infectious food-borne disease that was later known as the brucellosis due to *Brucella melitensis* and *Brucella abortus.* (4).

According to the international reports, Brucellosis (Malta fever) have the highest incidence in Iraq, Syria, Turkey, Mongolia, and Kyrgyzstan (5).

Human brucellosis also called Malta or undulant fever which is a serious public health problem has been reported all over the world. Common routes of infection include inoculation through cuts and abrasions in the skin or via the conjunctiva sac of the eyes, inhalation of infectious aerosols and ingestion via the gastrointestinal tract (6).

The hygienic quality of the raw milk produced in our country is not adequate for cheese production. However, due to the high demand from the consumers who prefer traditional and tasty cheese, the production of cheese from raw milk is still widespread. Almost at every stage of production technology in making cheese, there may be many risk factors causing microbial contamination (3,5).

Susceptibility to infection depends upon various factors, including the nutritional and immune status of the host, the size and route of the inoculum, and the species of *Brucella* causing the infection. Generally, *B. suis* and *B. melitensis* are more virulent for humans than are *B. abortus* and *B. canis* (7).

It has been reported that consumption of the cheese made from the milk of infected animals acts an important role in the transmission and spread of Brucellosis to human (8,9).

Despite the existing information about the disease, little has been reported about the prevalence of *Brucella ssp.* in dairy products in some cities of Iraq. Brucellosis is still an important infectious disease being widespread as endemic and sporadic cases in Iraq especially in Baquba city. Our aim in this study was to investigate the prevalence of *Brucella spp.* in local cheese " Gibin al Arab" at different street shops of the old town food markets in Baquba.

Materials and Methods

Sampling

A total of Fifty " Gibin al Arab" cheese sample was collected randomly in the periods from May 2015 till September 2015 in Diyala (Baquba) from retail sale premises of the old town of Baquba. Cheese samples were put into sterile plastic sample containers and were brought to the laboratory of Microbiology at Department of Pathological analysis, Baquba Technical Institute, Middle Technical University, Diyala under cold chain and were analyzed. Each sample was included of 100 g of local cheese.

Isolation and Diagnosis of *Brucella spp* . :

Identification of *Brucella* species were performed together with, colonial morphology, staining properties, serological screening and biochemical tests.

50 cheese specimens brought to the lab under aseptic condition, by using method of Marth .10g were taken from each cheese sample were added to 90ml of sterilized 2% sodium citrate then mixed by stomacher for about 5min. 1ml of each of this mixture inoculated to tubes containing 4ml of brain heart infusion broth (Difco), then incubated for 48hrs at 37˚C. After incubation 1ml of each tube was cultured by using *Brucella* selective agar (Himedia) plates and incubated at 37˚C for at least 5 days (10).Growth of bacteria were observed daily in these cultured plates. The colonies which have a property of *Brucella* were sub cultured for purification and identification. In selective serum agar, *Brucella ssp.* produces smooth, pinpoint, glistening and translucent colonies (6).

Identification of *Brucella* isolates

The isolates of bacteria were identified as *Brucella* species by using the conventional methods like, colony morphology and staining properties supported by applying motility test, Catalase test, Oxidase test, Urease test, Indol test, Lactose fermentation, Gelatin analysis and Blood haemolysis (11,12,13). To classify and biotype of *Brucella* species we used the following tests: Carbon dioxide requirement for growth, hydrogen sulfide production, dye sensitivity test (Viz basic fuchsine 1:50.000 and 1:100.000, thionin 1:25000, 1:50.000 and 1:100.000), Agglutination was observed by using slide agglutination test with monospecific antisera of *Brucella melitencis* (M) and *Brucella abortus* (A) (14, 15,16).

Results and Discussion

Brucellosis is an infectious zoonotic disease which infect animals naturally and is transmitted to humans by direct or indirect routes such as consumption of dairy products which are not pasteurized. Infection of *Brucella* caused by many species like almost, *B.melitensis* and *B.abortus*, caused by contact with infected animals (6).

Our results showed that out of fifty cheese samples collected only six *Brucella* isolates were found (at rates of 12%) this result agree with pamuk et al. who found *Brucella spp.* at rate of 14.2 % from collected aged skin bag cheeses samples (4), these results are approximately similar to kara et al. who isolate *Brucella spp.* in rate of 9% from white cheese samples (3), the rate of isolated *Brucella spp*. in the resent study is less higher from the results obtained by Abbas et al. (17) with the rate was 8% from collected cheese samples in Basrah city.

six *Brucella* isolated from cheese samples were distributed in to two species, four isolates were *B. melitensis* (at rate of 8%) and the rest were identified as *B. abortus* (at rate of 4%) , this agree with pamuk et al. obtained *B. melitensis* at rate 7% and *B. abortus* 2% in the analyzed fresh cheese samples (4), but not agree with Akbarmehr results which include isolation of *Brucella melitensis* at rate 7% and *B.abortus* 15% (2), The variation between the rates of *Brucella* spp. might be concluded from the differences in source of the milk used, the

level of the contamination, changes in production processes, use of raw milk, and production circumstances.

Brucella are Gram-negative, facultative intracellular bacteria that can infect many species of animals and man. They are sensitive to sunlight, disinfectant, pasteurization temperatures of 62.7C˚ for 30 min, or 71.6C˚ for 15 min, are sufficient to destroy the bacteria in milk. *Brucella* survive for several months at 4C˚ to 8C˚ in tap water and other liquid and are killed under freezing and thawing condition. *Brucella* do not survive in acidic condition like in sour milk or other low pH media (4,18).

The rate of *Brucella* spp. detected in this study was lower than the rates determined by Gorgi in study determined in Baghdad (19) the result was in rate of 60.6% from soft cheese, also the results of our research do not agree with Kerim who report 26 isolate of *Brucella spp.* among 60 sample of soft cheese in Tikrit province (20).

The reasons of contamination of cheese samples with *Brucella ssp.* may be due to secretion of milk from infected animal, from contaminated hands of workers, contaminated tool used in making cheese, dusts and flies or by using unpasteurized milk (21).

milking, transporting and offering for sale can develop contamination of the most consumed kind of white fresh cheese which called locally as Gibin Al-Arab with *Brucella ssp.*, which are produced and presented under improper hygienic conditions, cause infections or poisoning in human also it may be an important source of Brucellosis in our country which pose a threat to human health due to the increased number of cases and the severity of symptoms.

In order to eliminate brucellosis in Iraq, it must be pointed on the fact of limiting exposure to infection by using hygienic precautions and by effective heating of milk that used in making cheese. Boiling or pasteurizing milk and milk product effectively kills *Brucella* in dairy products for human consumption. Furthermore; education assumes an important role in preventing the transmission of brucellosis from animals to humans.

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