

DIRECT DETECTION OF MYCOBACTERIUM TUBERCULOSIS AND MYCOBACTERIUM BOVIS IN CATTLE RAW MILK BY USING SPECIES –SPECIFIC PCR TECHNIQUE

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ABSTRACT

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Fifty samples of raw cow milk were collected from clinically infected animals from different regions of Mosul city-Iraq, during the period between July to November, 2011 for direct detection of *Mycobacterium tuberculosis* and *Mycobacterium bovis* in the milk by using species-specific PCR technique. The Sacace MTB complex kit was used for DNA extraction and amplification from these samples. The Results showed that only four samples from fifty samples of suspected infected milk cow's were positive at ratio (8 %). These results ensured the occurrence of *M. tuberculosis* and *M. bovis* in the milk transmitted from animals to human and vice versa which may cause human tuberculosis, the study enhanced the significance of bacterial presence in raw milk and cause the infection to the consumer by drinking the contaminated or unpasteurized milk.

Key words: Cow milk, *M. tuberculosis* and *M. bovis*, PCR technique.

INTRODUCTION

Mycobacterium tuberculosis complex (MTC) are main causative agents of tuberculosis in many types of animals, birds and human. They are comprised of several types of species such as *Mycobacterium tuberculosis* causing human tuberculosis which is considered as one of the most important zoonotic disease and distributed throughout the world. and it is one member of *Mycobacterium tuberculosis* complex (Cole *et al.*, 1998; Alexander *et al.*, 2010). Bovine tuberculosis is also an important zoonotic disease which is caused by *Mycobacterium bovis*. The main reservoir of *M. bovis* infections is the infected cattle which served as the source of infection for human (Grange *et al.*, 1996). Bovine tuberculosis is easily transmitted from cattle to other and from cattle to human (Cousins and dawson *et al.*, 1999). Man usually get infection through contacts with the infected animals or by taking their contaminated raw milk and other milk products with the *M. bovis* bacteria (Ayele *et al.*, 2004; Collins and Grange *et al.*, 2003).

The human tuberculosis remains a major public health problem in the world, the rate of third and there are about 3 million human death annually (Cornejo *et al.*, 1998). The Nigerian human population carries the largest tuberculosis in Africa and it is of the fourth largest in the world which is estimated 390,000 human infections and 107,000 death per year (WHO, 1994). In 2004, the report of WHO showed that the mortality and morbidity statistics were 14.6 million chronic active cases, 8.9 million new cases and 1.6 million death. Most

infection cases occur in developing countries and the rate of infections increased were about 1% annually (WHO, 2006).

The disease in cattle is distributed in all the worldwide, there is no information about geographical boundaries of the disease. The clinical signs usually appear after one month of ingestion or inhalation of the bacilli and the disease appear when animals are exposed to stress or they appear in the old age. On the other hand, some cases of the infections can remain dormant for several years (Cousins, 2001).

The isolation of bacteria is highly specific but has poor sensitivity and it takes much time and intensive labor. Isolation of *M. bovis* via traditional culture methods may require 4–8 weeks (Sommer and Wood, 1985). There are several modern identification methods using DNA amplification techniques which have been developed for diagnosis of *Mycobacterium tuberculosis* complex (Smith *et al.*, 2006). Nucleic acid based on amplification techniques of which the Polymerase Chain Reaction has been so far the most extensively employed, offer several advantages over the classical microbiological methods such as shorter time of analysis, low detection limits, specificity and high sensitivity, thus PCR could be used as a screening technique (when performed at the herd level) or as a diagnostic technique (Kolk *et al.*, 1992; Vitale, 1998 and WHO, 2009).

Identification of the etiological agent belonging to MTC is important for determining the origin and reservoirs of infection and also for implementation of appropriate public health measures. The aim of this

study is to identify the incidence rate of *Mycobacterium tuberculosis* and *mycobacterium bovis* in raw milk of cattle in Nineveh governorate by using direct species-specific PCR technique which is considered a rapid method to detect the disease compared with the traditional method.

MATERIALS and METHOD

Fifty samples of raw cow's milk were collected from different regions of cattle farms in Mosul city during the period of July, to November, 2011, 10 ml of milk was collected in the sterile test tube and transport to the research lab of department of veterinary public health in the college of veterinary medicine, and stored in deep freeze at (- 20 C°) until used for DNA extraction process. For preparation the samples before extraction of DNA, 10 ml of milk sample is centrifuged at 3000 g/min for 10-15 min. The supernatant was discarded. If the pellet is not visible, add 10 ml of distilled water and repeat centrifugation and discard the supernatant. Resuspend the pellet in 100 µl of saline solution.

DNA extraction

Mycobacterium tuberculosis and *bovis* DNA was extracted using commercial DNA extraction kit (Sacace Biotechnology Srl, Italy). According to manufacture instructions prepare required quantity of 1.5 ml polypropylene tubes including one tube for Negative Control of Extraction. Add to each tube 10 µl of Internal Control and 300 µl of Lysis Solution. Add 100 µl of samples to the appropriate tube. Vortex the tubes and incubate for 5 min at 65°C. centrifuge all tubes for 30 sec at 8000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Add 300 µl of washing Solution 1 to each tube. Vortex vigorously and centrifuge for 30 sec at 8000g. Remove and discard supernatant from each tube. Add 500 µl of washing solution 2 to each tube. Vortex vigorously and centrifuge for 30 sec at 8000g. Remove and discard supernatant from each tube. Resuspend the pellet in 50 µl of DNA-eluent. Incubate for 5 min at 65°C and vortex periodically. Centrifuge the tubes for 1 min at maximum speed (12000-16000 g). The supernatant contains DNA ready for amplification. If amplification is not performed the same day of extraction, stored at frozen at 20°.

Amplification

Prepare required quantity of tubes PCR-mix-1. Pipette 10 µL of PCR-mix-2 into each PCR-mix-1 tube. Add to appropriate tube 10 µL of DNA sample obtained after sample preparation. Add 10 µL of DNA-buffer to the tube for negative control of amplification. Add 10 µL of positive control (dilute 1:10 with DNA buffer) to the tube for positive control of amplification. Close PCR-mix-1 tubes and transfer

them into the thermalcycler only when temperature reaches 105 °C and start the following program, one cycle at 95 °C for 5 min , 32 cycle (step 1: 95 °C for 30 s , step 2: 70 °C for 30s , step 3 : 72°C for 1 min), finally one cycle at 72 °C for 7 min, according to the Sacace MTB complex kit. Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (2%) staining by ethidium bromide. The length of specific amplified DNA fragments is 390 bp for *Mycobacterium tuberculosis* & *bovis* which visualized by using UV transilluminator and digital camera.

RESULTS and DISCUSSION

Milk and its products are regarded as the main source of nutrition to human being, on the other hand, they are considered as a good medium for many types of pathogenic bacteria and transmit the disease from animals to human through consumption the contaminated or unpasteurized milk (Sweeney *et al.*, 1992).

The result showed that only four samples from fifty milk samples collected from clinically infected cattle were positive and the incidence rate was 8%. Polymerase chain reaction (PCR) is considered as one of the most important modern technique in the world for being rapid, sensitive, and the practical technique which based on the amplification of highly specific DNA sequences present in the agent of interest in different samples by giving amplified bands weighted 390 bp when electrophoresed on 2 % agarose gel staining with ethidium bromide (Tiwari *et al.*, 2007), showed in figure (1).

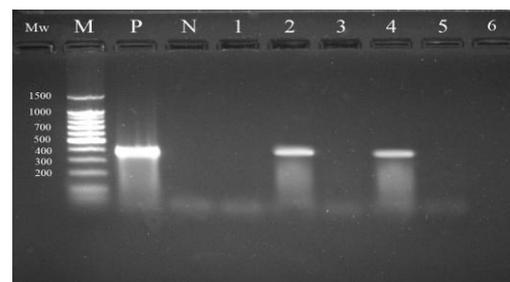


Figure (1): PCR amplification results of *M. tuberculosis* and *bovis* appeared at 390 bp. M : DNA Ladder, P : positive control, N: negative control, 2 and 4 amplified fragments for *Mycobacterium spp.* were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide, 1,3,5 and 6 negative for *Mycobacterium spp.*

The incidence rate obtained from this study is similar to the previous study (Sulieman *et al.*, 2008) which showed the rate of infection is (8.64 %), while the rate of incidence of our study is less than the previous study (Ameni *et al.*, 2007; Naima *et al.*, 2008; Al-Saquer *et al.*, 2009 and Gobena, 2010) which are recorded (46.8%), (29 %), (10.2 %) and (53.6 %)

respectively, the high incidence rate of the previous studies is attributed to the collection of the samples from infected cattle herds, and also from the developing countries, they do not have any effective programs to prevent the occurrence of the disease in the cattle herd (Cassidy, 2006), while the rate of infection of our study is high from (Ofukwu *et al.*, 2008) which recorded (1.4 %) this is attributed to use the conventional method to detect the Mycobacterium tuberculosis complex that it is commonly used in the developing countries to identify the infected animals (Gilbert *et al.*, 2005).

The type of the method used to detect mycobacterium tuberculosis complex plays a major role in detection of the disease between the cattle herd. In developing countries that use the conventional method to detection instead of tuberculin skin test and selective culture media. Mycobacteria grow slowly and the growth of these bacteria appeared after several weeks and the result was confirmed by biochemical test to detect the mycobacterium (Palmer and Waters, 2006). Therefore, we must use rapid and sensitive method to reach accurate diagnosis because administration of the antibiotic without taking any recommendation from veterinarians is very dangerous and it may cause an increase in the resistance of mycobacterium tuberculosis complex to antibiotic by sequential acquisition of chromosomal mutation (Sebastien *et al.*, 2006).

Although the cattle included in the studied areas are not reared intensively, but the source of infection is expected due to lack of control and eradicate programs of the disease in Iraq as well as mismanagement, lack of veterinary care, lack of biosecurity program and malnutrition, where cattle leave for long period outside the barns with bad rations, pushing the cattle grazing in waste contaminated areas and as well as connection with other domestic and wild animals, all of these factors may lead to increased exposure to the disease (Al-Saqur, 2009).

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الكشف المباشر عن عصيات السل البشري وعصيات السل ألبقري في الحليب الخام للأبقار باستخدام تقنية النوع المتخصص لتفاعل البلمرة المتسلسل

رعد عبد الغني السنجري ، عمر هاشم شيت ، ضياء محمد طاهر جوهر

جمعت (50) عينة حليب خام من أبقار مصابة سريريا من حقول الأبقار في مناطق مختلفة من مدينة الموصل- العراق، للفترة ما بين شهر تموز إلى شهر تشرين الثاني من عام 2011 وذلك للكشف المباشر عن تواجد جراثيم الـ *Mycobacterium tuberculosis* و *Mycobacterium bovis* في الحليب باستخدام تقنية تفاعل البلمرة المتسلسل من النوع المتخصص. استخدم في هذه الدراسة طقم خاص بعزل وتضاعف الحامض النووي لعصيات السل والمجهز من شركة ساكاسي الإيطالية. أظهرت نتائج الدراسة أن أربعة عينات من حليب الأبقار كانت موجبة لعصيات السل اي بنسبة 8 %، من خلال نتائج الدراسة تبين تواجد جراثيم *M. tuberculosis* و *M. bovis* في الحليب الخام للأبقار المصابة واحتمالية انتقالها إلى الإنسان في حالة تناول الحليب الملوث بهذه الجراثيم مسببة مرض السل للإنسان وكذلك إمكانية انتقالها إلى الحيوان ، ومن خلال الدراسة تبين أهمية تواجد هذه الجراثيم الخطرة في حليب الأبقار المصابة وانتقالها إلى الإنسان عن طريق تناول الحليب الغير معقم.