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Prevalence of Brucella Organism in Milk and Serum Samples of Some Lactating Dairy Animals

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ABSTRACT

Brucellosis is a worldwide zoonotic disease that cause major public health hazard in Egypt. The present work was conducted to investigate the prevalence of bovine brucellosis on the basis of serological test RBPT and MRT in serum and milk samples of lactating dairy animals, also isolation of brucella organism from milk samples, PCR amplification was done for detection of Brucella abortus DNA using bcsp-31 target gene. 219 serum samples from sheep and goat and 119 milk samples from cow, sheep and goat were collected from governmental and private farm. The prevalence of brucellosis by RBPT were 34.5 and 61.4% in serum samples of sheep and goat while by MRT were 2.33, 13.8 and 10% in milk samples of cow, sheep and goat, respectively. The same prevalence was obtained when milk samples were cultured on the Brucella agar medium. A specific PCR assay targeting the *bcsp-31* gene within the genome of Brucella species has been used and gave an amplicon of 223 bp in three strain of isolated brucella. It is concluded that RBPT and MRT used as screening tests for detection the prevalence of brucella species in serum and milk samples, PCR assay consider as a useful tool for detection Brucella species in milk.

1. INTRODUCTION

The health of milk is highly important for the consumers. Thousands of people worldwide are infected with bacteria through milk each year that cause infection and food poisoning (Najwa et al., 2011). There is a wide variety of bacteria in milk, that one of them is brucella (Ragona et al., 2016) Brucella is a small gram-negative bacterium, facultative intracellular, highly aerobic and fastidious, which cause brucellosis in cows, sheep, goats and humans (zoonotic disease in humans and animals) (Azar et al., 2006).

Brucellosis is a highly contagious bacterial zoonosis affecting livestock and humans worldwide and has different names: Infectious or enzootic abortion and Bang's disease in animals; and Mediterranean or Malta fever, Crimean fever, Undulant fever and Rock fever in humans (Xavier and Paixao, 2010). Brucella infection cause abortion, stillbirth, mastitis, metritis, and placental retention in females and orchitis and arthritis in males. Infertility may be seen in both sexes. The true incidence of human brucellosis is not easy to estimate globally, but an estimated 500,000 persons are newly infected every year (Neubauer, 2010). Brucellosis can be transmitted either by direct contact with infected animals and animal excreta or indirect contact through ingestion of contaminated milk containing large quantities of bacteria (Chen et al., 2014).

Milk ring test (MRT) is a field test used for diagnosis and detection of brucella antibodies in milk and considered principle methods for detecting infected herds and diagnosis of brucella in individual animal (Noriello 2004). Rose Bengal plate test (RBPT) is based on agglutination of colored particulate antigen (killed Brucella organisms) by the

antibodies present in the patient's serum (Chachra et al., 2009).

Uses of sensitive and rapid diagnostic methods for detection of brucella infection are important. At present, the most common methods for diagnosis brucella in live dairy cattle involves either the isolation of Brucella organism from milk samples or the detection of anti-Brucella antibodies in milk (Hamdy and Amin, 2002).

Molecular methods for brucellosis detection are faster and more sensitive than traditional methods, but the sensitivity and specificity of PCR tests may be vary among laboratories (Navarro et al., 2004). Recently, polymerase chain reaction (PCR)-based detection of organisms has been found to be more convenient as compared to cultural isolation. PCR is an option for brucellosis diagnosis; however, a few studies have been carried out with field samples in a way to use the reaction as a diagnostic tool (O'Leary et al., 2006). A number of nucleic acid sequences have been targeted for the development of Brucella genus specific PCR assays, including 16S rRNA and bcsp-31 (Leal-Klevezas et al., 1995).

With consideration of the above facts the present study was carried out to investigate the prevalence of brucellosis on the basis of serological test such as Rose Bengal Plate Test (RBPT) in serum samples of sheep and goat, MRT in milk samples of some lactating animals including cow, sheep and goat. Also, application of conventional PCR as sensitive tool for detection of brucella species using bcsp31k gene specific

2. MATERIAL AND METHODS

1. Materials:

1.1. Samples collection:

Collection of 219 serum samples from sheep and goat animal in eppendorf tube as follow (136 samples from sheep and 83 from goat) randomly from unvaccinated animals as well as from apparently healthy animals of different age groups from governmental and private farms at June 2016. Also, collection of 121 milk samples (43 from cow, 58 from sheep and 20 samples from goat) has been collected from governmental and private farms. Milk samples were collected in sterile jars (500 ml. capacity), then transferred to the laboratory in an insulated ice box with a minimum of delay, where directly examined.

2. Methods:

2.1. Rose Bengal Plate Agglutination (OIE, 2012): serum samples were collected and stored at-20°C until they were assayed. Equal volume of both brucella colored antigen and serum sample were mixed, definite agglutination was taken as positive reaction, whereas no agglutination as negative.

2.2. Milk Ring Test (MRT) (**Alton et al., 1988):** The test was performed by adding 30 µl of brucella antigen to 1 ml of whole milk that had been stored for at least 24 h at 4 °C. The height of the milk column in the tube was at least 25 mm. A positive reaction was indicated by formation of a blue ring above a white milk column or at the interface of milk and cream. The test was considered to be negative if the color of the underlying milk exceeded that of the cream layer.

2.3. Isolation of brucella organism (**Alavi and Motlagh, 2012):** Milk samples were quite homogeny and kept in refrigerator for an hour. Centrifugation 5 ml of each milk samples at 3500 rpm, cream layer was cultured in brucella agar medium supplied with antibiotic supplement then milk was culture after cream. Plate incubated in anaerobic jar because of the CO2 production at 35-37°C for 48 hours. small colonies white to gray, non-mucoid colonies consider positive results, Considering the slow growth of Brucella, they were kept up to 1 week for ensuring the negative sample of brucella.

2.4. Polymerase chain reaction for detection of brucella:

2.4.1. DNA extraction: From biochemical identified brucella species, Four DNA was extracted using proteinase K and sodium dodecyl sulfate method. The DNA was purified twice with phenol-chloroform using Phase Lock Gel Heavy tubes (Eppendorf AG, Hamburg, Germany). The DNA was also precipitated and washed, and the pellet was resuspended in 50 µL of nuclease-free water. Two hundred nanograms of DNA template were used afterwards for PCR amplifications.

2.5. Primer sequences used for PCR identification system of brucella:

Detection of the gene encoding Brucella cell surface protein (*bcsP31*) which encodes an immunogenic membrane protein of a 31 kDa antigen of B. abortus and is conserved in all Brucella biovars (Baily et al., 1992).

Primer sequence used in PCR

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Reference
bBcs-P31 (F)	**5′** ACGCAGTCAGACGTTGCCTAT **′3**	**223**	**Guarino et al. (2000)**
bcs-P31 (R)	**5′** TCCAGCGCACCATCTTTCAGCCTC **′3**		

3. RESULTS AND DISCUSSION

Table (1): Prevalence of brucella organism in serum samples of sheep and goat using rose Bengal plate test.

Animals	No. of serum samples	RBPT9a			
		Positive		Negative	
		No	%	No	%
Sheep	**136**	47	34.55	89	65.44
Goat	**83**	51	61.4	32	38.6

Table (2): Prevalence of brucella organism in cow milk samples using MRT and culture.

Test	No of examined samples	Positive		Negative	
		No	%	No	%
MRT	**43**	1	2.33	42	97.76
Brucella culture	**43**	1	2.33	42	97.76

Table (3): Prevalence of brucella organism in sheep milk samples using MRT and culture.

Test	No of examined samples	Positive		Negative	
		No	%	No	%
MRT	**58**	8	13.8	50	86.2
Brucella culture	**58**	8	13.8	50	86.2

Table (4): Prevalence of brucella organism in goat milk samples using MRT and culture.

Test	No of examined samples	Positive		Negative	
		No	%	No	%
MRT	**20**	2	10	18	90
Brucella culture	**20**	2	10	18	90

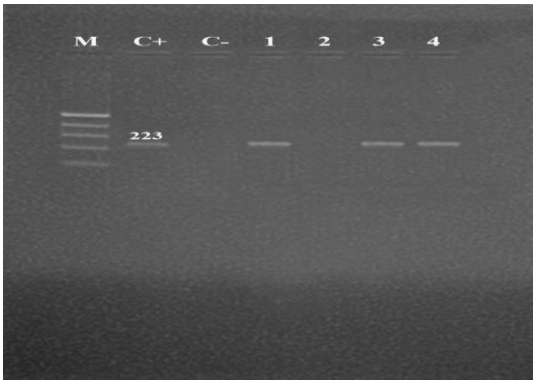

Figure (1): Agarose gel electrophoresis of PCR amplification Products of *bcsp-31* gene specific for identification of *Brucella species*. **Lane M:** 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive *Brucella species* for *bcsp31k* gene. **Lane C-:** Control negative.
Lanes 1, 3 and 4: Positive *Brucella species* strains for *bcsp31k* gene. **Lane 2:** Negative *Brucella species* strain.

Brucellosis is still considered as the serious health problems in human and domestic animals. It transmitted to human by drinking contaminated raw milk from infecting lactating dairy animals.

In present study, Table (1) showed that RBPT was positive in 34.55% of sheep (47 out of 136 serum samples), while in goat serum samples was positive in 61.4% (51 out of 83 samples). Therefore, RBPT is considered to be important for primary detection of individual animals infected with brucellosis even if the antibody levels However, some cross-reacting antibodies have been detected by this test and hence false negative reaction may notice (OIE, 2012).

In another study which carried out in India (Padher et al., 2017), examined 200 serum samples (100 from goats and 100 from sheep) for presence of brucella antibodies using RBPT. They found that in goat,16 % of samples were found seropositive while in sheep 11 % of samples were found seropositive by RBPT.

Yousif (2010) tested 300 serum samples from cattle in Sudan with RBPT and found that 31 (10.3%) samples were positive, 100 samples from goats were tested with RBPT and found 6 % were positive (6 samples out of 100 samples), finally examined 100 serum samples from sheep and found that 7 % were positive (7 samples out of 100 samples).

The obtained results in Table (2) showed that brucella antibodies were present in cow milk. based on screening test by MRT, the prevalence found in percent 2.33% and these results confirmed by isolation of brucella organisms from the same samples and found that organism was isolated from one sample (2.33%). Lower incidence recorded in present study might due to passive immunization of calves through colostrum of their infected dams (Mohammed et al., 2011).

Higher result was recorded by (Gogoi et al., 2017), who reported the prevalence of brucella antibodies found in cattle ranged from 0.00 to 18.75% with an overall prevalence of 10.53%. Also, Rezaei et al. (2010) in Iran, found that the prevalence rate for B. abortus infection in cow using MRT was 14%. Yousif (2010) examined 200 cattle milk samples in Khartoum and found that 23 samples (11.5%) were positive for MRT.

Although, MRT is considered as an ideal method for detecting infected cow and diagnosis of brucellosis in individual animals (Noriello, 2004), it may be giving false positive reactions in colostrum or milk at the end of the lactation period and milk from cows suffering from a hormonal disorder or mastitis (Bercovich and Moerman, 1979).

Regarding results in Table (3), the prevalence rate of brucella antibodies in sheep milk using MRT was 13.8% (8 samples out of 58 samples), isolation of brucella from the same samples proved that incidence rate was 13.8%.

Data presented in table (4) showed that prevalence rate of brucella infection in goat milk samples based on MRT was 10 % (2 samples out of 20 samples), confirmation of the result by isolation brucella organism from the same samples indicated that only two samples (10 %) were positive for brucella infection.

Although, WHO (1992) reported that MRT is not suitable for sheep and goats as ring formation do not readily occur but Ali (2014) examined 120 suspected milk samples from goats with history of abortion that were collected from Al-Samawa city, Iraq and tested with MRT. He found that 11 samples (9.16%) were positive for MRT.

For PCR, species-specific primer for brucella abortus infection have been investigated, 31-kDa (*bcsp*-31K) antigen that encodes a 31-kDa protein has proved to be a promising target because of the presence of species-specific signature regions in the gene (Matar et al., 1996). In the past there has been considerable interest in the use of rapid molecular biology-based assays to identify and determine the species of brucella (Bricker and Halling, 1995; Corbel, 1997).

The PCR assay in Figure (1) showed that an amplicon of 223 bp was obtained in three species of the tested sample using genus-specific primer derived from a gene encoding the 31-kDa Brucella species antigen. Al-Mariri and Nermeen, (2010) recommended use of PCR procedure to identify brucella in all types of milk samples.

Al-Mariri (2015) examined PCR assay for detection Brucella genus in Syrian bovine milk samples using specific primers that amplify a conserved region in Brucella. Agarose gel electrophoresis of the *bcs31* conventional PCR amplification gave a product with size of 223 bp, indicating the presence of Brucella genus in milk sample.

Conclusion and Recommendation

Based on the outcome of this study, it is obvious that serological test such as MRT and RBPT

were used as screening tests for Brucella antibodies in serum and milk, respectively in lactating dairy animals. The finding indicated sensitivity and specificity of PCR assay as useful tool for diagnosis of Brucella species in milk.

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