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Molecular Detection of *Brucella* Organism from Milk and Milk Products

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A B S T R A C T

Brucellosis is the most common zoonosis in the world, Brucellosis is an infectious disease caused by bacteria of the genus *Brucella.* The disease has a considerable impact on human and animal health, as well as socioeconomic impacts, especially in which rural income relies largely on livestock breeding and dairy products. The disease can also be transmitted by direct or indirect contact with infected animals. Humans are commonly infected through ingestion of raw milk, milk product, meat or through direct contact with infected animals. In presence study was undertaken for the molecular detection of *brucella* organism from milk and milk products from different places of Gujarat. Out of 85 milk samples, 23 (27.05%) samples were found positive for *Brucella* antibodies by Milk Ring Test (MRT). A total of 168 samples (145 milk product and 23 MRT positive milk samples) processed for detection of *Brucella* organism using genus specific (B4/B5 primer) PCR. Out of 168 samples, 14 samples amplicon at 223bp by *Brucella* genus specific B4/B5primers indicates that all fourteen samples positive for *Brucella* organism.

Introduction

Brucellosis is an important re-emerging zoonosis with a worldwide distribution. It is still an uncontrolled serious public health problem in many developing countries including India (Mantur *et al.*, 2007). According to the food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organization of Animal Health (OIE) brucellosis considered to be the most widespread zoonosis throughout the world (Mustafa, 1995). Brucellosis is an infectious disease caused by Gram negative, facultative, intracellular bacterial organisms of the genus *Brucella* that is pathogenic for a wide variety of animals and human beings. It causes significant reproductive losses in sexually mature animals (Forbes, 1996; Wadood *et al.*, 2009). The disease is manifested by late term abortions, weak calves, stillbirths, infertility and characterized mainly by placentitis, epididymitis and orchitis, with excretion of the organisms in uterine discharges and milk (England *et al.*, 2004). The commonest source of *Brucella* infection is the dairy cow and the commonest mode of transmission is the ingestion of milk and milk product containing viable *Brucella*. Thus the identification of

milk from infected cow is a matter of substantial public health importance.

Diagnosis of Brucellosis by cultural isolation, serology and nucleic acid amplification has been explored for the techniques. A number of nucleic acid sequences have been targeted for the development of *Brucella* genus specific PCR assays, including 16S rRNA (Romero *et al.,* 1995), IS711 genetic element, omp2 (Leal-Klevezas *et al.,* 1995) and bcsp31.

Rapid detection and confirmation of *Brucella*, the Brucellosis diagnosis, surveillance and screening by various serological tests, milk ring test is one of the most important screening test for detection of *Brucella* antibody from milk. Confirmatory diagnosis of brucellosis is by molecular level.

Materials and Methods

Collection of samples

A total of 230 samples of milk and milk products (cheese, paneer, curd, ice-cream, cream) were collected in a sterile container from various places of Gujarat (Table 1).

Milk Ring Test (MRT) / Aborts Bang ring test (ABR)

After thorough mixing 3 ml milk samples were taken in a test tube. Add 3 drops of ABR antigen and gently mixed (ABR-antigen prepared byIAH and VB, Hebbal, Bangalore). The tubes were incubated at 37^{o}C for one hour. Then keep at room temperature for 30 min.

Blue colored ring of cream layer at the top and absence of color in milk layer is considered as a positive. If whole milk retains blue is considered as a negative.

Molecular detection of *Brucella*

DNA extraction

To make the suspension of milk product using homogenizer, DNA extraction was carried out from milk product (145) and MRT positive milk (23) samples using DNeasy Blood and Tissue Kit (Qiagen) following manufacturers protocols.

Detection of *Brucella* using genus-specific B4/B5 primer

A PCR was standardized in a total reaction volume of 25 µl, containing 12.5 µl of 2 x PCR Master mixture, 10 pmol of forward (5'TGG CTC GGT TGC CAA TAT CAA3') and reverse (5'CGC GCT TGC CTT TCA GGT CTG3')[4]primers each 1 µl, Template DNA 2 µl and nuclease free water upto 25 µl. The reaction was standardized in a thermal cycler (Eppendorf, Germany). with initial denaturation at 93ºC for 5 min, followed by 35 cycles at 90ºC for 60 s, 64ºC for 30 s and 72ºC for 60 s. Final extension was carried out at 72ºC for 10 min. The amplified product (223bp) was electrophoresed in 2% agarose gel stained with ethidium bromide (0.5 µg/ml) and image was documented by gel documentation system (Mini BiS Bio Imaging System).

Results and Discussion

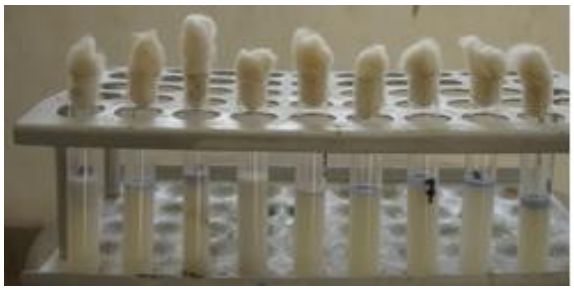
Milk ring test

Out of 85 milk samples, of these 23 (27.05%) of samples were found positive for *Brucella* antibodies by MRT (Fig. 1). Present finding was in agreement with earlier studies which detected 25.21% *Brucella* antibody from milk by MRT (Zowghi *et al.*, 1990). However, In contrast to the present findings, reported 9.16% (Al-Mariri, 2015) and 56.32% (Gulluce, 1996) *Brucella* antibody from milk by MRT.

Table.1 Collection of samples from different places

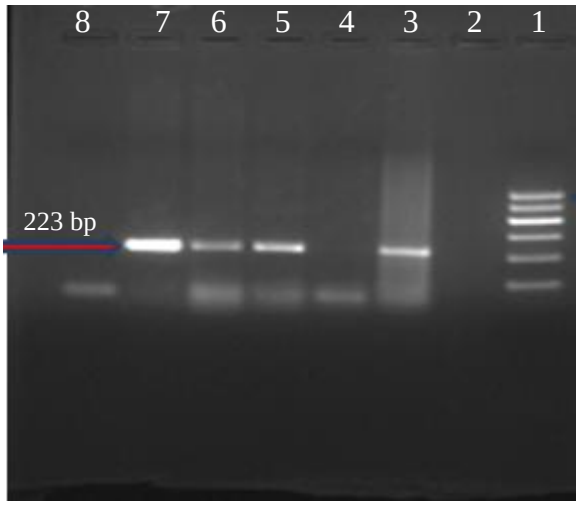
Sr. No.	Type of samples	Places/Locations					Total
		Local vendor	Deesa	Palanpur	Dantiwada	Patan	
1.	Milk	45	10	10	10	10	**85**
2.	Curd	10	5	5	5	5	**30**
3.	Cheese	---	5	5	5	5	**20**
4.	Ice-cream	5	10	10	10	10	**45**
5.	Paneer	5	5	5	5	5	**25**
6.	Butter	5	5	5	5	5	**25**
Total		**70**	**40**	**40**	**40**	**40**	**230**

Fig.1 Milk ring test



MRT Negative MRT Positive

Fig.2 Genus specific PCR



1-ladder 2-Negative control
3- Positive control 4- Sample (negative)
5-Sample (positive) 6- Sample (positive)
7- Sample (positive) 8- Sample (negative)

Molecular Detection of *Brucella*

In PCR study targeting 16S rRNA gene, Out of 168 samples fourteen samples were found positive to give specific amplicon of 223bp region of the sequence encoding a 31 kDa immunogenic bcsp31 by *Brucella* genus specific primer pairs B4/B5 (Fig. 2). Similarly, Kanani (2007) and Jung *et al*., (1998) detection of *Brucella* by using bcsp31 gene based B4/B5primer. Similarly, Ali (2014), Al-Mariri (2015) and Akbarmehr (2011) also detect *Brucella* organism from milk and milk products.

In conclusion, the detection of *Brucella* organism from milk of *Brucella* affected animal which is of public health importance because it is zoonotic disease. There is need to educate about how to prevent and control of brucellosis transmitted from animal to human by milk and milk product.

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