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# Clinico-Seroepidemiology and Molecular Characterization of Brucellosis in Animals

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**A B S T R A C T**

The present study reports clinico seroepidemiological observations of Brucellosis including molecular epidemiology. During the period of study a total of 6410 sera were collected, which included cattle (2723), buffaloes (894), sheep (1072), goats (1281), camel (438) and equine(02). These sera were screened for the presence of *Brucella* antibodies by RBPT and i-ELISA. The overall seroprevalence detected in animals were 12.85% (824 out of 6410) and 11.87% (761 out of 6410) by RBPT and i-ELISA, respectively. Clinical conditions wise seroprevalence was recorded in heifer 4.65% and 6.97%, clinically healthy animals 7.23% and 6.56%, animals with the history of abortion 25.58% and 29.09%, hygroma 13.33% and 11.66%, pregnant 5.45% and 4.21%, non-pregnant 7.81% and 6.17%, status unknown 7.74% and 6.16%, still birth 16.00% and 20.00%, retention of placenta 14.22% and 11.71%, repeat breeding 19.70% and 14.35%, orchitis 34.34% and 18.18%respectively by RBPT and i-ELISA. Out of 744 milk samples of aborted animal, 152 milk samples found positive for *Brucella* antibody by MRT. A total l110 clinical samples were processed by genus specific PCR using B4/B5 (223bp) primer. Of these 15 samples positive for *Brucella* organism. Out of 15 genus specific PCR positive samples, 12samples positive by *B. abortus* +IS711 (498bp) species specific PCR and 3 samples positive by *B. melitensis omp31* (723bp) species specific PCR. All the 15 samples also confirmed positive by Bruce ladder PCR.

## Introduction

Brucellosis is caused by various species of the genus *Brucella*, which is the second most widely spread zoonosis worldwide (Dawood, 2008). *Brucella* can affect almost all domestic species and cross transmission can occur between cattle, sheep, goat, camel and other species (Ghanem *et al.,* 2009). Brucellosis is characterized by abortion and birth of non-visible offspring in females, orchitis and epididymitis in males (Radostits *et al.,* 2007) (Fig. 1) It may also cause chronic inflammation of joints, tendon sheath and synovial bursa especially at the carpus (Abbas and Agab, 2002). There are many factors that can affect the prevalence of brucellosis in various species of livestock. Prevalence may vary according to climatic conditions, geography, species, sex, age and diagnostic tests used (Gul and Khan, 2007). Proper treatment and prevention of disease requires

prompt and accurate diagnosis. Molecular diagnostics provides excellent platform for accurate and prompt diagnosis of diseases while maintaining safety of the personnel (Sola *et al.,* 2014) and detection of *Brucella* organisms in various clinical, blood and serum samples of livestock and human employing PCR, Real time PCR, speciation by species specific PCR and Bruce ladder multiplex PCR. The disease has been reported from various species in different parts of India. Therefore, present study was planned for overall seroprevalence and molecular epidemiological characteristics of Brucellosis in animals.

## Materials and Methods

### Seroprevalance

A total 6410 Serum samples Cattle (2723), Buffaloes (894), Sheep (1072), Goats (1281), Camels (438), and equine (02) were collected from various district of Gujarat for detection of *Brucella* antibodies.

### Rose Bengal Plate Test (RBPT)

One drop (30 µl) of known *Brucella* antigen (IAHVB, Hebbal, Bangalore) was taken on a glass slide by micropipette then add equal amount of suspected serum samples and mixed thoroughly. The result was read immediately. Definite clumping/agglutination was considered as positive reaction, whereas no clumping/agglutination was considered as negative.

### Indirect ELISA

Indirect ELISA kit was procured from National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) Bangalore, and used as per manufacturer's protocols. The kit detects the antibodies against *Brucella* lipopolysaccharide (LPS) in serum samples of *B. aborus* and *B. melitensis.*

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

A total of 744 milk samples were collected in sterial vial 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 by IAHVB, 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*

A total of 1110 various clinical samples were collected from cattle, buffaloes, sheep, goats, camel and equine for molecular characterization of *Brucella.*

### DNA extraction

The genomic DNA from clinical samples was extracted using DNeasy Blood and Tissue Kit (Qiagen, USA) following manufacturers protocols.

### Detection of *Brucella* using Genus-Specific, Species-specific and Bruce-ladder PCR

These samples processed for genus PCR, species specific PCR and Bruce-ladder PCR for confirmation using primer pair (Table 1) and conditions of thermal cycling for different primer pairs in PCR (Table 2) and PCR reaction mixture was prepared as per table 3.

PCR amplified product was checked by running samples over 2.0% agarose gel.

## Results and Discussion

### Seroprevalence

Out of 6410 sera sample, overall seroprevalence recorded in animals was

12.85% (824 out of 6410) and 11.87% (761 out of 6410) by RBPT (Fig. 2) and i-ELISA (Fig. 3) respectively (Table 4). Aulakh *et al.,* (2008) reported over all seroprevalence of 18.26% in cattle and buffaloes from Punjab by ELISA. Gumber *et al.,* (2004) reported over all seroprevalence of 22.5% positive in cattle and buffaloes in Punjab, by RBPT. Panchasara *et al.,* (2015) reported overall seroprevalence was 10.66%, 10.29% and 9.38% by RBPT, STAT and i-ELISA, respectively in North Gujarat. However, in contrast to the present study higher rate of overall seroprevalence 28.00%was reported by Ahmed *et al.,* (2010) by RBPT. Kushwaha *et al.,* (2015) also reported very high seroprevalence rate of 33.85%, 32.61% and 30.90 % by ELISA, RBPT and STAT, respectively in Pakistan. Clinical symptoms wise seroprevalence was 4.65% to 34.34% by RBPT and 4.21% to 29.09% by i-ELISA (Table 5).

**Table.1** List of primers

| **Genus specific primers:** | | | |
|---|---|---|---|
| Primer Forward/ Reverse | Sequence (5'-3') | Product size (bp) | Reference |
| B4 (F) | TGG CTC GGT TGC CAA TAT CAA | 223bp | Bailey *et al.,* (1992) |
| B5 (R) | CGC GCT TGC CTT TCA GGT CTG | | |
| **Species specific primers *(B.abortus):*** | | | |
| IS711(F) | GAC GAA CGG AAT TTT TCC AAT CCC | 498 bp | Bricker and Halling, (1994). |
| IS711(R) | TGC CGA TCA CTT AAG GGC CTT CAT | | |
| **Species specific primers *(B.melitensis):*** | | | |
| Omp31(F) | TGACAGACTTTTTCGCCGAA | 723bp | Vizcaino *et al* (1996) |
| Omp31(R) | TATGGATTGCAGCACCGC | | |
| **Bruce Ladder Primers:** | | | |
| BMEI0998 (F) | ATCCTATTGCCCCGATAAGG | 1682bp | Lopez-Goni*et al.,* (2008) |
| BMEI0997(R) | GCTTCGCATTTTCACTGTAGC | | |
| BMEI0535(F) | GCGCATTCTTCGGTTATGAA | 450bp | |
| BMEI0536(R) | CGCAGGGGAAAACAGCTATAA | | |
| BMEII0843(F) | TTTACACAGGCAATCCAGCA | 1071bp | |
| BMEII0844(R) | GCGTCCAGTTGTTGTTGATG | | |
| BMEII436(F) | ACGCAGACGACCTTCGGTAT | 794bp | |
| BMEII435(R) | TTTATCCATCGCCCTGTCAC | | |
| BMEII0428(F) | GCCGCTATTATGTGGACTGG | 587bp | |
| BMEII0428(R) | AATGACTTCACGGTCGTTCG | | |
| BR0953(F) | GGAACACTACGCCACCTTGT | 272bp | |
| BR0953(R) | GATGGAGCAAACGCTGAAG | | |
| BMEI0752(F) | CAGGCAAACCCTCAGAAGC | l218bp | |
| BMEI0752(R) | GATGTGGTAACGCACACCAA | | |
| BMEII0987(F) | CGCAGACAGTGACCATCAAA | 152bp | |
| BMEII987(R) | GTATTCAGCCCCCGTTACCT | | |

**Table.2** Steps and conditions of thermal cycling for different primer pairs in PCR

| Primers (Forward and Reverse) | Cycling conditions | | | | | |
|---|---|---|---|---|---|---|
| | **Initial denaturation** | **Denaturation** | **Annealing** | **Extension** | **Final extension** | **Cooling** |
| B4 (F)<br>B5 (R) | 93°C<br>5 min | 90°C<br>1 min | 64°C<br>30 sec | 72°C<br>1 min | 72°C<br>10 min | 4 °C |
| | **1 cycle** | **Repeated for 35 cycles** | | | **1 cycle** | |
| IS711(F)<br>IS711(R)<br>*(B. abortus)* | 95°C<br>2 min. | 95°C<br>1.15 min. | 55.5°C<br>2 min. | 72°C<br>2 min. | 72°C<br>2 min. | |
| | **1 cycle** | **Repeated for 35 cycles** | | | **1 cycle** | |
| Omp31(F)<br>Omp31(R)<br>*(B. melitensis)* | 94°C<br>5 min. | 94°C<br>1 min. | 58°C<br>1 min. | 72°C<br>2 min. | 72°C<br>10 min. | |
| | **1 cycle** | **Repeated for 35 cycles** | | | **1 cycle** | |
| Bruce Ladder Primers | 95°C<br>7 min | 95°C<br>35 second | 64°C<br>45 sec | 72°C<br>3 min | 72°C<br>6 min | |
| | **1 cycle** | **Repeated for 25 cycles** | | | **1 cycle** | |

**Table.3** Various components used in PCR

| **Components** | **Genus/ Species specific PCR** | **Components** | **Bruce ladder PCR** |
|---|---|---|---|
| PCR Master Mix (2X) | 12.5 µl | PCR Master Mix (2X) | 12.5 µl |
| Forward Primer (10 pmol/µl) | 1 µl | Bruce-ladder eight pair primer cocktail (12.5µM) | 1 µl |
| Reverse Primer (10 pmol/µl) | 1 µl | | |
| Template DNA | 2 µl | Template DNA | 2 µl |
| Nuclease free water | 8.5 µl | Nuclease free water | 8.5 µl |
| **Total** | **25 µl** | **Total** | **25 µl** |

**Table.4** Overall seroprevalence

| **Species** | **No. of tested** | **Seroprevalance** | |
|---|---|---|---|
| | | **RBPT positive** | **i-ELISA positive** |
| **Cattle** | 2723 | 333(12.22%) | 307(11.27%) |
| **Buffalo** | 894 | 129(14.42%) | 116(12.97%) |
| **Goat** | 1281 | 123(9.60%) | 108(8.43%) |
| **Sheep** | 1072 | 170(15.85%) | 171(15.95%) |
| **Camel** | 438 | 69(15.75%) | 59(13.47%) |
| **Equine** | 02 | 00(0.00%) | 00(0.00%) |
| **Total** | **6410** | **824(12.85%)** | **761(11.87%)** |

**Table.5** Clinical status wise seroprevalence

| Symptoms | No. of tested | Seroprevalance | |
|---|---|---|---|
| | | RBPT positive | i-ELISA positive |
| **Heifer** | 172 | 08(4.65%) | 12(6.97%) |
| **Clinically healthy** | 2087 | 151(7.23%) | 137(6.56%) |
| **Abortion** | 1110 | 284(25.58%) | 323(29.09%) |
| **Hygroma** | 180 | 24(13.33%) | 21(11.66%) |
| **Pragnant** | 403 | 22(5.45%) | 17(4.21%) |
| **Non-pragnant** | 486 | 38(7.81%) | 30(6.17%) |
| **Status unknown** | 762 | 59(7.74%) | 47(6.16%) |
| **Still birth** | 50 | 08(16.00%) | 10(20.00%) |
| **Retension of Placenta** | 239 | 34(14.22%) | 28(11.71%) |
| **Repeat breeding** | 822 | 162)19.70%) | 118(14.35%) |
| **Orchitis** | 99 | 34(34.34%) | 18(18.18%) |
| **Total** | **6410** | **824(12.85%)** | **761(11.87%)** |

**Table.6** Processing of milk samples by MRT

| Species | No. of tested | MRT positive |
|---|---|---|
| **Cattle** | 584 | 116(19.86%) |
| **Buffalo** | 154 | 36(23.37%) |
| **Camel** | 06 | 00(0.00%) |
| **Total** | **744** | **152(20.43%)** |

**Fig.1** Clinical symptoms

**Table.7** Direct detection of Brucella by PCR

| Type of sample | Species | | | | | | No.of tested | Result |
|---|---|---|---|---|---|---|---|---|
| | Cattle | Buffalo | Sheep | Goat | Camel | Equine | | No. of sample positive in PCR |
| **Blood** | 231 | 102 | 178 | 111 | 91 | 09 | 722 | 00 |
| **Vaginal swab** | 73* | 45* | 37 | 31 | 03 | 02 | 191 | **02** |
| **Vaginal discharge** | 08** | 05* | 02* | 00 | 00 | 00 | 15 | **04** |
| **Milk** | 12 | 08 | 04 | 06 | 03 | 00 | 33 | 00 |
| **Placenta** | 17** | 09** | 04* | 01 | 00 | 00 | 31 | **05** |
| **Placental fluid** | 05 | 02 | 00 | 00 | 00 | 00 | 07 | 00 |
| **Hygroma fluid** | 02 | 01 | 00 | 00 | 00 | 00 | 03 | 00 |
| **Amniotic fluid** | 00 | 02 | 00 | 00 | 00 | 00 | 02 | 00 |
| **Orchitis fluid** | 00 | 00 | 03 | 01 | 00 | 00 | 04 | 00 |
| **Foetal intestine** | 00 | 00 | 03 | 01 | 00 | 00 | 04 | 00 |
| **Foetal intestine fluid** | 02 | 01 | 01 | 02 | 00 | 00 | 06 | 00 |
| **Foetal lung** | 09* | 04 | 02 | 06 | 00 | 00 | 21 | **01** |
| **Foetal liver** | 09* | 04 | 02 | 06 | 00 | 00 | 21 | **01** |
| **Foetalabomasal content** | 00 | 00 | 03* | 02 | 00 | 00 | 05 | **01** |
| **Foetal stomach content** | 03* | 01 | 03 | 05 | 00 | 00 | 12 | **01** |
| **Foetal heart** | 03 | 00 | 02 | 02 | 00 | 00 | 07 | 00 |
| **Foetal heart blood** | 06 | 04 | 02 | 04 | 00 | 00 | 16 | 00 |
| **Cotyledon** | 06 | 04 | 00 | 00 | 00 | 00 | 10 | 00 |
| **Total** | **386** | **192** | **246** | **178** | **97** | **11** | **1110** | **15 Sample** |

*indicate number of positive sample in PCR

**Fig.2** Rose Bengal test

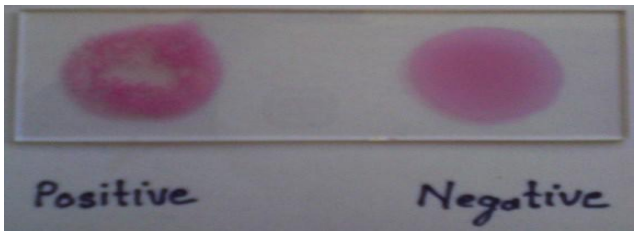
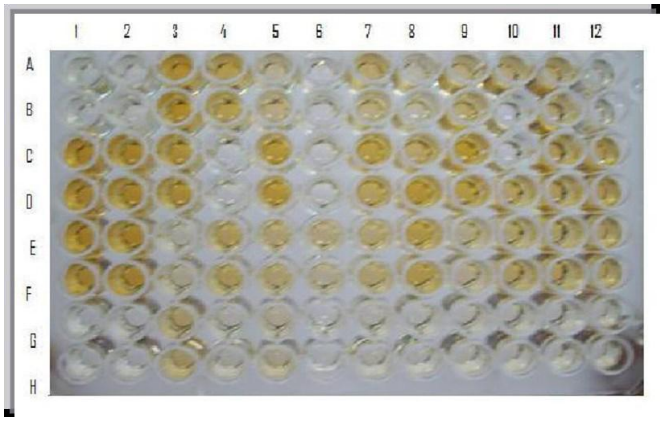


**Fig.3** i-ELISA




Wells A1, B1, A2, B2: Negative control; Wells C1, D1, C2, D2: Moderately positive control
Wells E1, F1, E2, F2: Strong positive control; Wells A3, B3, C3, D3, C5, D5 etc. positive field sera reaction

**Fig.4** Milk ring test

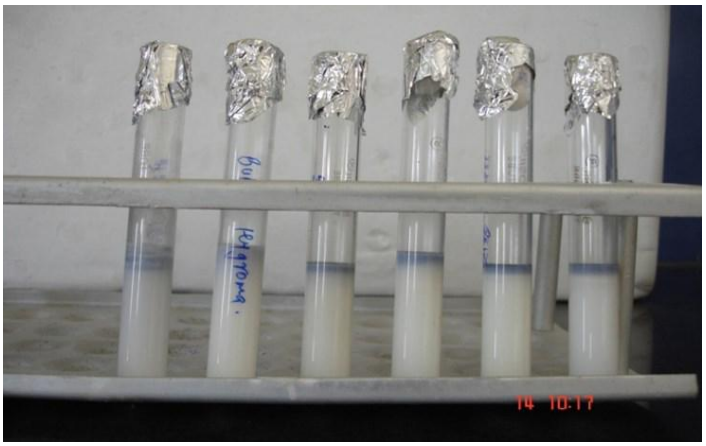
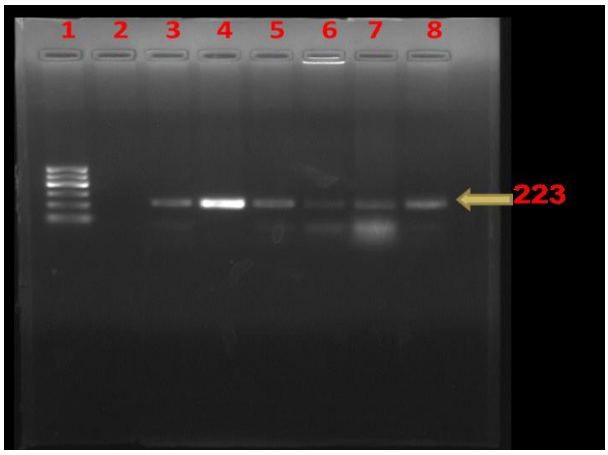
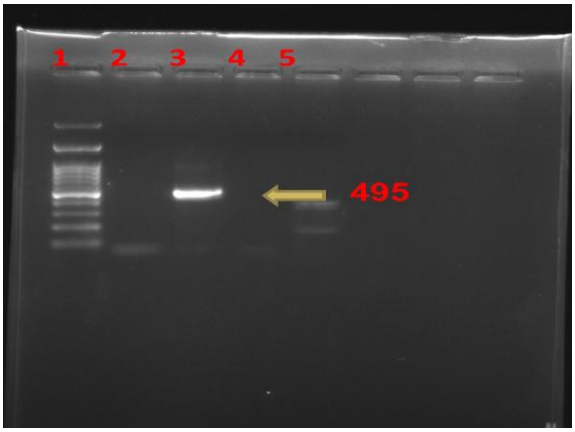


**Fig.5** Genus specific PCR223bp PCR products with B4B5 primer



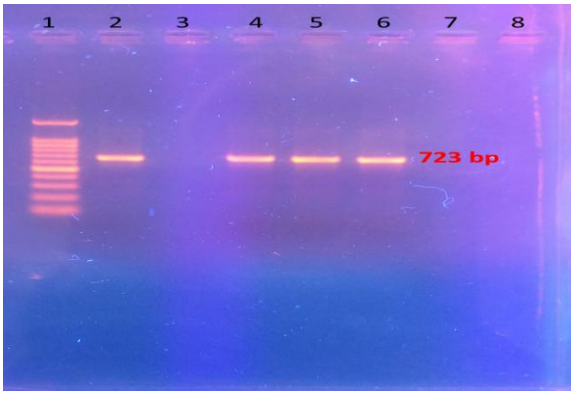

1-ladder
2-NTC
3- sample (positive)Vaginal discharge
4- sample (positive) Foetal lung
5-sample (positive)Placenta
6- sample (positive)Foetal liver
7- sample (positive)Foetal stomach content

**Fig.6** Species specific PCR495 bp PCR product with IS711 primer



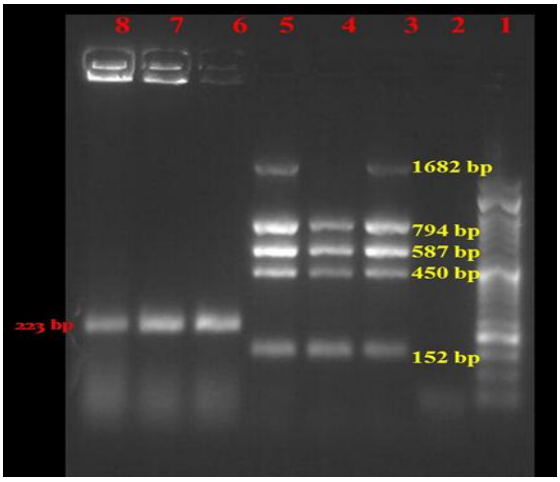

1-ladder
2-NTC
3- sample (positive)Foetal lung
4- sample (negative)
5-sample (negative)

**Fig.7** Species specific PCR 723bp PCR product with omp31 primer




1- Ladder
2-Positive control
3- NTC
4-Sample (positive) vaginal discharge
5- Sample (positive) fetal abomasal content
6- Sample (positive) placenta

**Fig.8** Bruce ladder PCR




1- Ladder
2-NTC
3- Sample (positive)Placenta (Bruce ladder)
4-Sample (positive) vaginal discharge (Bruce ladder)
5- Sample (positive) vaginal swab (Bruce ladder)
6- Sample (positive) placenta (Genus Specific)
7- Sample (positive) vaginal discharge (Genus Specific)
8- Sample (positive) vaginal swab(Genus Specific)

Out of 744 milk samples, of these 152 (20.43%) samples were found positive for *Brucella* antibodies by MRT (Fig. 4; Table 6). Present finding was in agreement with Zowghi *et al.*, (1990) which detected 25.21% *Brucella* antibody from milk by MRT. However, In

contrast to the present findings, Al-Mariri (2015) reported 9.16%and Gulluce *et al.,* (1996) reported 56.32% *Brucella* antibody from milk by MRT.

## Molecular detection of *Brucella* from clinical samples

In PCR study targeting 16S rRNA gene, Out of 1110 clinical samples fifteen samples (Table 7) 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. 5). Out of 15 genus specific positive samples, 12 samples yielded an amplicon of 498bp in *+IS711* primers indicate species as *Brucella abortus* (Fig. 6) and 3 samples yielded an amplicon of 723bp in *omp31* primers indicate species as *Brucella melitensis* (Fig. 7). All 15 genus specific positive samples were also confirmed as positive by Bruce ladder PCR (Fig. 8). Similarly, Kanani (2007) and Jung *et al.*,(1998) detection of *Brucella* by using bcsp31 gene based B4/B5primer.Earlier Navarro *et al.*, (2002), Kanani (2007) and Patel (2007) used same primer pairs for molecular detection of *Brucella abortus.* Patel *et al.,* (2015) and Karthik *et al.,* (2014) used species specific +IS711 primers for detection of *Brucella abortus* and they yielding 498bp band and Vizcaino *et al.,* (1996)used species specific omp31 primers for detection of *B. melitensis* and they yielding 723bp band when electrophoresed through 2% agarose gel. Lopez Goni *et al.,* (2008) reported Bruce-ladder was species specific and all the strains and biovars from the same *Brucella* species gave the same profile.

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## References

Abbas, B. and Agab, H. 2002. A review of camel brucellosis. *Preventive Vet. Med.*, 55: 47-56.

Abouzeed, Y.M., Ibrahim, A., Salem, H., Alzwam, F., Abid, S., Elfahem, A. and Elrais, A. 2010. Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006–January 2008. *Euro Surveill.*, 15(30): 19625.

Al-Mariri. 2015. Isolation of *Brucella melitensis* strains from Syrian bovine milk samples. *Bulgarian J. Vet. Med.*, 18(1): 40.

Aulakh, H.K., Patil, P.K., Sharma, S., Kumar, H., Mahajan, V. and Sandhu, K.S. 2008. A Study on the Epidemiology of bovine brucellosis in Punjab (India) using milk-ELISA. *Acta Veterinaria Brno*., 77: 393–399.

Bailey, G.G., Krahn, J.B., Drasar, B.S. and Stoker, N.G. 1992. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *J. Trop. Med. Hygiene*, 95: 271-275.

Bricker and Halling. 1994. Differentiation of *Brucella abortus* bv. 1, 2 and 4 *Brucella melitensis*, *Brucella ovis* and *Brucella suis* bv 1 by PCR. *J. Clin. Microbiol.*, 32: 2660-2666.

Dawood, H.A. 2008. Brucellosis in camels (*Camelusdro medorius*) in the south province of Jordan. *Am. J. Agric. Biol. Sci.*, 3: 623-626.

Ghanem, Y.M., El-Khodery, S.A., Saad, A.A., Abdelkader, A.H., Heybe, A. and Musse, Y.A. 2009. Seroprevalance of camel brucellosis (*Camelisdro medarus*) in Somaliland. *Trop. Anim. Health Prod.*, 41: 1779-1786.

Gul, S.T. and Khan, A. 2007. Epidemiology and Epizootology of Brucellosis: A Review. *Pak. Vet. J.*, 27:145-151.

Gulluce, M. and Leloglu, N. 1996. Detection of *Brucella abortus* antibodies in cow milk of the Kars area by ELISA and MRT. *Turkish J. Vet. Animal Sci*., 20(4): 251-255.

Gumber, S., Aradhana, A., Dhand, N.K. and Sandhu, K.S. 2004. Village level study of bovine brucellosis in Punjab (India) by

bulk milk analysis. *Indian J. Animal Sci.*, 74: 843-844.

Jung, S.C., Jung B.Y., Woo, S.R., Cho, D.H., Kim, J.Y., Kim, W.T., Lee, J.M., Park, Y.H. and Baek, B.K. 1998. Development of a PCR assay for the detection of *Brucella* spp. in bovine semen. *Korean J. Vet. Res.*, 38: 345-352.

Kanani, A.N. 2007. Serological, cultural and molecular detection of *Brucella* infection in breeding bulls. Ph. D. thesis submitted to A. A. U., Anand.

Karthik, K., Rathore, R., Thomas, P., Arun, T.R., Viswas, K.N., Agarwal, R.K., Manjunathachar, H.V. and Dhama, K. 2014. Loop-mediated isothermal amplification (LAMP) test for specific and rapid detection of *Brucella abortus* in cattle. *Vet. Quarterly*, 34(4): 174-179.

Kushwaha, N., Rajora, V.S., Mohan, A., Upadhyay, A.K. and Kumar, R. 2015. Comparison of serological tests for detection of *Brucella* antibodies in cattle of an organized dairy farm. *Indian J. Animal Res.*, 50(1): 69-74.

Lopez-Goni, I., Garcia-Yoldi, D., Marin, C.M., Miguel, M.J., Munoz, P.M., Blasco, J.M., Jacques, I., Grayon, M., Cloeckaert, A., Ferreira, A.C., Cardoso, R., Correa de Sa, M.I., Walravens, K., Albert, D. and Garin-Bastuji, B. 2008. Evaluation of a multiplex PCR assay (Bruce-ladder) for molecular typing of all *Brucella* species, including the vaccine strains. *J. Clin. Microbiol.*, 46: 3484-3487.

Navarro, E., Escribano, J., Fernandez, J. A. and Solera, J. 2002. Comparison of three different PCR methods for detection of *Brucella* spp. in human blood samples. *FEMS Immunol. Med. Microbiol.*, 34: 147-151.

Panchasara, H.H., Patel, J.S. and Patel, P.R. 2015. Seroepidemiological pattern of brucellosis among buffaloes in north Gujarat. *Indian J. Vet. Sci. Biotechnol.*, 11(1): 74-79.

Patel, B.C., Chauhan, H.C., Chandel, B.S., Dadawala, A.I. and Jain., B.K. 2015. Seroprevalence and Molecular characterization of *Brucella* spp. in buffalo from North Gujarat, India. *Int. J. Curr. Microbiol. Appl. Sci.*, 4(4): 174-180.

Patel, T.J. 2007. Serological, cultural and molecular detection of *Brucella* infection in bovines including quantification in milk by real-time PCR. An M.V. Sc. thesis submitted to A. A. U., Anand.

Radostits O.M., Gay C., Blood D.C. and Hinchclift K.W. 2007. Disease associated with helminthes parasite. In: veterinary medicine, a text book of the disease of cattle, sheep, goat, pigs and horses. 10th ed. London: Bailliere, Tindall, UK, 984-988.

Sola, M.C., Da Veiga, J.E.A., De Freitas, M.R. and De Mesquita, A.J. 2014. Real-time PCR detection of *Brucella spp.* DNA in lesions and viscera of bovine carcasses. *J. Microbiol. Methods*,104: 87-91.

Vizcaino, N., Cloeckaert, A., Zygmunt, M.S. and Dubray, G. 1996. Cloning, nucleotide sequence and expression of omp31 gene coding for an immunogenic major outer membrane protein. *Infect. Immunity*, 64(9): 3744-3751.

Zowghi, E., Ebadi, A. 1990. Mohseni, B. Isolation of *Brucella* organisms from the milk of seronegative cow. *Rev. sci. Tech. Off. Int. Epiz.*, 9(4): 1175-1178.

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