



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2017; 5(6): 1436-1440

© 2017 JEZS

Received: 10-09-2017

Accepted: 12-10-2017

Ameen-ur-Rashid

Dept. of Biosciences, COMSATS
Institute of Information
Technology, Islamabad,
Pakistan

Muhammad Asif Gondal

Dept. of Biosciences, COMSATS
Institute of Information
Technology, Islamabad,
Pakistan

Abid Ali Khan

Dept. of Biosciences, COMSATS
Institute of Information
Technology, Islamabad,
Pakistan

Anwar Ali

Veterinary Research Institute,
Peshawar, Pakistani

Mirza Ali Khan

Veterinary Research Institute,
Peshawar, Pakistani

Rafiullah

Veterinary Research Institute,
Peshawar, Pakistani

Siraj Ahmed

Veterinary Research Institute,
Peshawar, Pakistani

Inamullah Wazir

Institute of Microbiology,
University of Agriculture,
Faisalabad, Pakistan

Said Sajjad Ali Shah

The University of Agriculture,
Peshawar, Pakistani

Sohail Khan

Veterinary Research Institute,
Peshawar, Pakistani

Correspondence

Ameen-ur-Rashid

Dept. of Biosciences, COMSATS
Institute of Information
Technology, Islamabad,
Pakistan

Molecular and serological study of caprine and ovine brucellosis in district Peshawar

Ameen-ur-Rashid, Muhammad Asif Gondal, Abid Ali Khan, Anwar Ali, Mirza Ali Khan, Rafiullah, Siraj Ahmed, Inamullah Wazir, Said Sajjad Ali Shah and Sohail Khan

Abstract

Brucellosis is an infectious disease of livestock and wild animals and is the second most important zoonotic disease after rabies throughout the world. Brucellosis in small ruminants is mainly caused by *B. melitensis* and in bovines it is caused by *B. abortus* while *B. melitensis* is also the causative agent of brucellosis in humans. This study was designed to investigate the prevalence of brucellosis in small ruminants of District Peshawar. A total of 300 blood samples (150 each from sheep and goats) were randomly collected and screened through Rose Bengal Plate Test (VRI and UK Antigens), ELISA and PCR. Over all prevalence was 4.33% through RBPT (VRI), 3.67% through RBPT (UK) and ELISA while 3.3% through PCR. Species wise prevalence of brucellosis was higher in goats as compared to sheep but no statistical significance ($P>0.05$) was recorded. Similarly, brucellosis was noted comparatively higher in female but statistically no significant relation was observed. Reproductive tract of the female is the potential reservoir of bacteria and can easily propagate and cause disease. Mal husbandry practices, overcrowding and use of brucellosis affected or carrier male animals for breeding purpose may lead to a higher prevalence of brucellosis in animals. Results of the study revealed that serological tests i.e. Rose Bengal Plate Test and ELISA are reliable and can be used in combination with PCR for confirmation of brucellosis.

Keywords: Brucellosis, Small ruminants, Zoonotic, Rose Bengal Plate Test, ELISA, PCR

1. Introduction

Brucellosis is a bacterial, infectious and zoonotic disease caused by *Brucella*, which is a gram negative, non-motile, intracellular cocco-bacilli [1]. This disease is also known as Malta fever, Undulant fever, Mediterranean Fever and Bang's disease. It is considered as second most important zoonotic disease worldwide after rabies [2, 3]. There are different species of *Brucella* which cause brucellosis in different domestic and wild animals. In small and large ruminants, brucellosis is caused by *B. melitensis* and *B. abortus*, while in humans; *Brucella melitensis* is the main etiological agent [4]. *Brucella* is classified as a class B bioterrorist agent. Five lac cases are annually reporting worldwide [5].

Brucellosis causes severe reproductive disorders and important clinical signs and indications include abortion, arthritis, fetal membrane retention, orchitis, epididymitis, metritis, temporary/permanent infertility, weak and immunosuppressed off-springs [6]. It primarily affects veterinarians, abattoir laborers, livestock producers and farmers, shepherds and laboratory workers/technicians [7]. It is endemic in many regions globally such as Northern and Eastern Africa, Latin America, Eastern Europe, Mediterranean Region, Middle East, Caribbean Region and South Asia [8]. Brucellosis is prevalent in Pakistan and work has been carried out on prevalence of the disease in different animals and has been reported from 0-32.5 % [9].

There is a huge population of small ruminants (Sheep 29.8 million and goats 70.3 million) in Pakistan, especially in Balochistan and northern regions of Khyber Pakhtunkhwa. In Pakistan almost 8 million families are involved in Livestock sector and they depend on livestock for their livelihood. Keeping in view this huge population, Pakistan has the potential to export milk, meat, skin, hides and wool to other countries of the world. But due to lack of diagnostic facilities and disease control measures, export of animal products is not up to the level. Different diseases of zoonotic and contagious nature in small ruminants, including Brucellosis possess serious trade embargo and thus limiting the export of animal products [10].

Different diagnostic tests i.e. Rose Bengal Plate Test, Serum Agglutination Test, ELISA are usually suggested for screening animals flocks/herds and individual animals^[11]. Isolation of *Brucella* organism from blood, bone marrow and cerebrospinal fluid is the gold standard for diagnosis of brucellosis but has some limitations^[12]. Different conventional diagnostic tests are used for the identification of brucellosis with variable success because of lack of specificity and sensitivity. To overcome the limitations of other techniques, molecular test i.e. PCR is used for the diagnosis of *Brucella* species, as it is rapid, sensitive and highly specific^[13]. Keeping in view the importance of brucellosis, this study was intended with the objective to find out the prevalence of brucellosis in district Peshawar by using conventional test (Rose Bengal Plate test), serological test (ELISA) and molecular test (PCR).

This study was intended to document the prevalence of brucellosis in caprines and ovines, as they are an essential part of livestock; by using a combination of serological and molecular diagnostic techniques including Rose Bengal Plate Test (RBPT) with Veterinary Research Institute Lahore Antigen, RBPT with Lillidale United Kingdom Antigen, ELISA and PCR in Peshawar, Pakistan. These three techniques are never used before combine in Pakistan. This study is a base line to take the initiative of eradication and control program against brucellosis.

2. Materials and Methods

2.1. Study Area

The study was conducted in district Peshawar, capital of Khyber Pakhtunkhwa. Peshawar features a semi-arid climate, very hot summers and mild winters. District Peshawar is located at 4.00°N 71.32°E, consists of an area of 1257 kilometer square and is 359 m above mean sea level.

2.2. Sample collection

A total of 300 samples of blood (150 each from goat and sheep) were collected from both male and female animals randomly having no abortion and vaccination history. Blood samples were collected in EDTA tubes for DNA extraction and gel tubes for serological examination of brucellosis. All the samples were processed in Brucellosis Laboratory, Veterinary Research Institute, Peshawar.

2.3. Rose Bengal Plate Test (RBPT)

Serum samples were exposed to Rose Bengal Plate Test for the detection of antibodies against *Brucellosis* through RBT antigen of Veterinary Research Institute Lahore and RBT antigen of the Lillidale United Kingdom. The samples were processed as procedure said by the manufacturers and processed according to Gurturk *et al.* (1999). An aliquot of 30µl of serum sample along with 30 µl of RBT antigen was placed on the agglutination plate through micropipettes with separate tips for each sample and were mixed by an applicator. The glass plate was rotated clockwise for 4 minutes and seen under indirect light source. Agglutination indicates positive sample.

2.5. ELISA

Serum that was isolated from the blood samples was subjected to i-ELISA to detect antibodies against *Brucellosis*.

Indirect ELISA for brucellosis was performed according to the standard protocol of IDEXX Switzerland.

2.6. PCR

Genomic DNA was extracted from anticoagulant added whole blood through DNA extraction kit according to the manufacturer instruction (NucleoSpin® Genomic DNA from tissue and blood). PCR amplification of extracted DNA was done by using *Brucella* genus specific primers as described by Rabab *et al.* (2000). Total of the 25µl reaction mixture was prepared by adding 4 µl of Master Mix (Solis biodyne), 0.5 µl of forward primer and 0.5 µl of reverse primer, 3 µl of template DNA and 17 µl of ddH₂O. Positive control and negative control samples were also processed along with field samples. PCR reaction conditions were; initial denaturation at 94°C for 2 min followed by 35 cycles of denaturation at 93°C for 15sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec and final extension at 72°C for 10 min. Gel electrophoresis of PCR product was performed by using 1.2% agarose gel, 110v, 500mA for 30 min and finally gel was observed in UV gel documentation system (Multi-gene Labnet International Inc. USA). Following the *Brucella* genus specific primer was used for amplification of DNA.

Forward Primer-5'-TGGCTCGTTGCCAATATCAA-3'
Reverse Primer-5'-CGCGCTTGCCTTCAGGTCTG-3'

The clear bands of *Brucella* genome at 223bp were considered as positive results^[14].

2.7. Statistical Analyses

Data was arranged in MS excel and analyzed statistically for significance through the Chi-square test by using SPSS 19.0.

3. Results

3.1. Overall prevalence of Brucellosis

Samples were screened through RBPT by using VRI, Lahore antigen and Lillidale (UK) antigen. Out of total 300 samples tested, 13 samples (4.33%) were positive on VRI, Lahore antigen while 11 (3.67%) samples showed positive reaction to Lillidale (UK) antigen (Fig I). Similarly, 13 samples (4.33%) were found positive through Indirect ELISA (Fig 2) while 10 samples (3.33%) were positive on PCR (Fig III) (Table I).

3.2. Species wise prevalence of Brucellosis

Prevalence of brucellosis in goats was recorded as 5.34% on RBPT (VRI antigen) while 4.67% prevalence was recorded on RBPT (UK antigen), ELISA and PCR. In sheep prevalence was recorded as 3.3% on RBPT (VRI antigen), 2.67% on RBPT (UK antigen) and ELISA while 2% prevalence was observed on PCR. Specie wise prevalence was slightly higher on RBPT (VRI antigen) but no statistical significance ($P>0.05$) was observed with respect to other tests. (Table II).

3.3. Sex wise prevalence of Brucellosis

In female, brucellosis was recorded as 5.33% on RBPT and ELISA while through PCR it was observed as 4.67%. In male, 3.3% animals showed positive reaction on RBPT (VRI antigen) while 2% animals were declared positive on RBPT (UK antigen), ELISA and PCR. Prevalence of brucellosis on RBPT (VRI antigen) was bit higher but no significant relation ($P>0.05$) was observed with other techniques used (Table III).

Table 1: Overall prevalence of Brucellosis in small ruminants in district Peshawar.

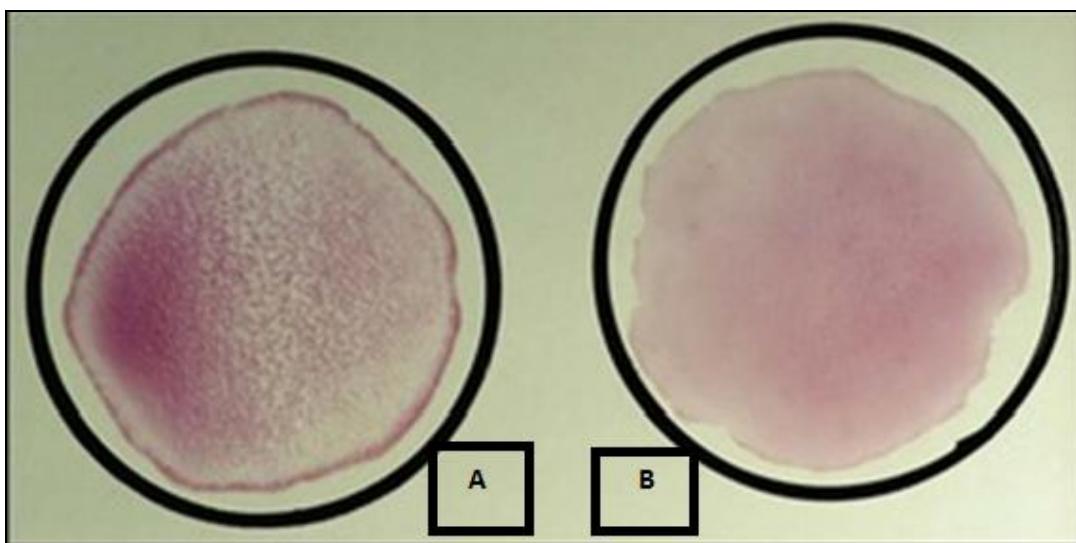
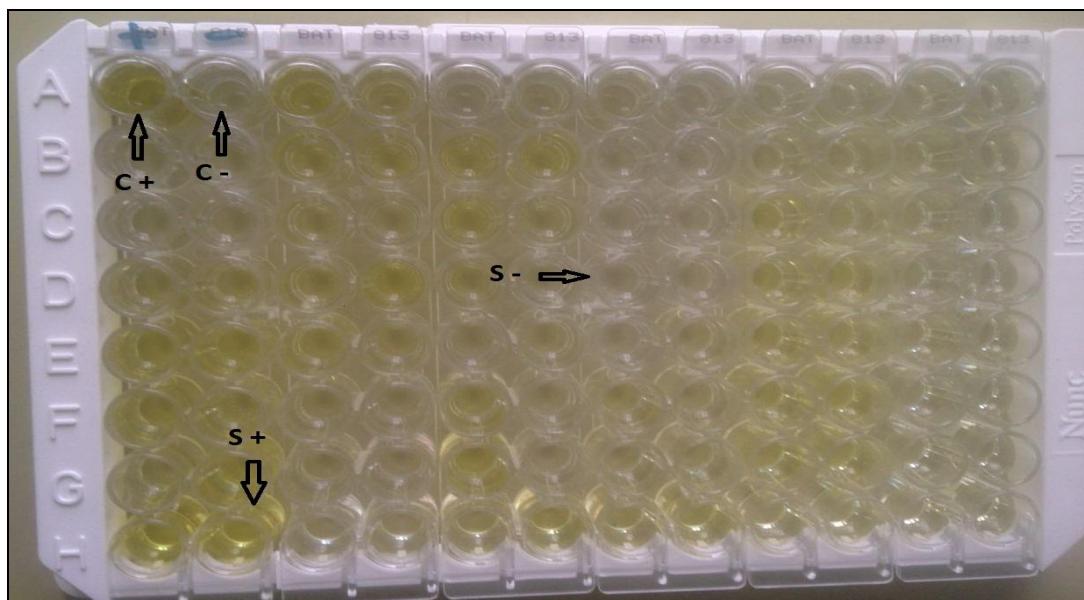
Tests	No. of positive cases	% prevalence	P-value
RBPT (VRI antigen)	13/300	4.33	0.179
RBPT (UK antigen)	11/300	3.67	
ELISA	11/300	3.67	
PCR	10/300	3.33	

Table 2: Specie wise prevalence of brucellosis in small ruminants of Peshawar.

Tests	Goats		Sheep		P-value
	N	% P	N	% P	
RBPT (VRI)	8/150	5.34	5/150	3.3	0.28
RBPT (UK)	7/150	4.67	4/150	2.67	
ELISA	7/150	4.67	4/150	2.67	
PCR	7/150	4.67	3/150	2	

Table 3: Sex wise prevalence of brucellosis in sheep and goats in district Peshawar

Tests	Female		Male		P-value
	N	%P	N	%P	
RBPT (VRI)	8/150	5.33	5/150	3.33	0.390
RBPT (UK)	8/150	5.33	3/150	2	
ELISA	8/150	5.33	3/150	2	
PCR	7/150	4.67	3/150	2	

**Fig 1:** Rose Bengal plate test results, A shows agglutination (Positive sample) B shows no agglutination (Negative sample)**Fig 2:** ELISA plate showing (C+) control positive, (C-) control negative, (S+) sample positive and (S-) sample negative

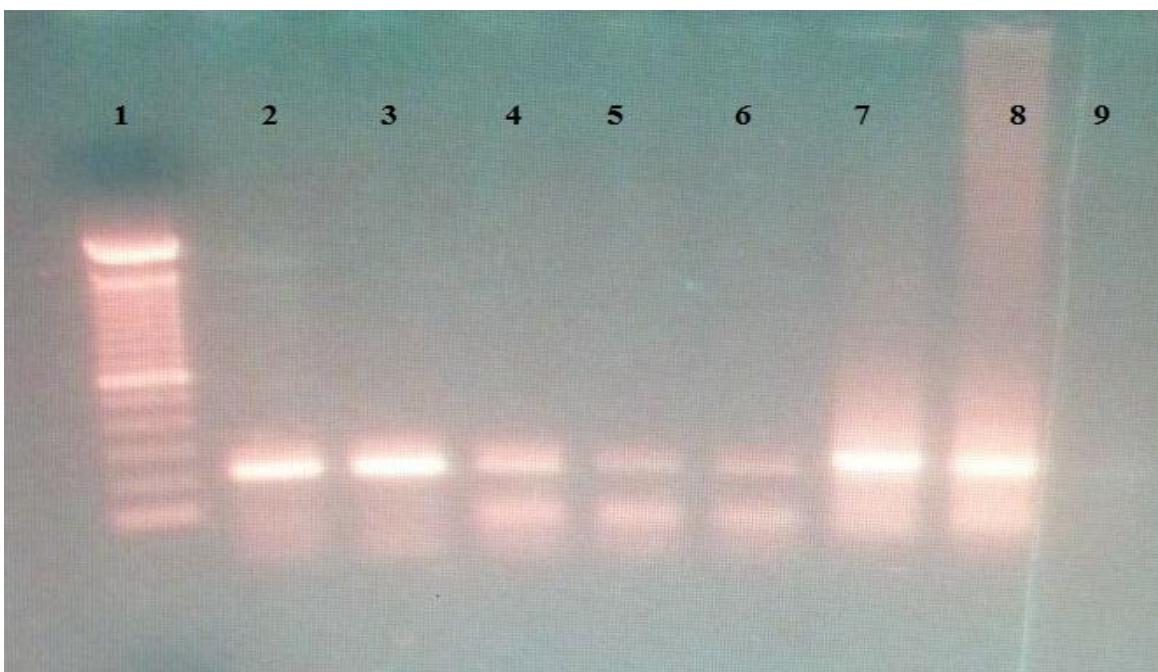


Fig 3: PCR result showing (1) 100 bp DNA marker, (2) 223 bp Brucella positive control (3-7) Positive samples showing bands at 223bp (8) Positive control, (9) Negative control.

4. Discussion

Brucellosis is highly communicable and infectious disease of small ruminants, having zoonotic and economic importance [15]. It has adverse effects on production and reproduction potential of small ruminants in terms of fall or complete termination of milk production followed abortion, loss of off springs/young ones and temporary/permanent sterility [16]. Overall brucellosis prevalence in the current study was 4.33% through RBPT (VRI antigen), 3.67% through RBPT (UK antigen) and ELISA while 3.33% prevalence was recorded through PCR. Hussain *et al.* (2014) reported the same results by using RBPT (VRI antigen) while studying the prevalence of brucellosis in district Kohat [17]. District Peshawar and Kohat are in close vicinity to each other and same management practices are used in both districts which might be the possible reason for the same results. Slightly higher prevalence of brucellosis was recorded by Ali *et al.* (2015) in small ruminants of Pothohar region [18]. Ali *et al.* (2015) collected blood and milk samples from small ruminants which were in close contact with seropositive bovine herd. Brucellosis is a contagious disease and can easily spread due to close contact of animals with each other and this might be the possibility of higher prevalence of brucellosis from the current study [19].

Most of the researchers used various diagnostic tools to detect brucellosis in small ruminants. Prevalence was stated slightly higher by various researchers by using RBPT while through ELISA and PCR, prevalence was comparatively lower. Similar pattern of prevalence was observed in the current study, with 4.33% through RBPT while 3.67 and 3.33% through ELISA and PCR, respectively. PCR is the technique that is more specific and sensitive technique for the accurate detection of even minute quantity of *Brucella* antigen as reported by many researchers [20, 21]. Prevalence through i-ELISA in the current study was observed slightly lower from the study conducted by Rajala *et al.* (2016) who stated 6.7% prevalence of brucellosis in small ruminants in Tajikistan. This slight variation in prevalence might be due to difference in sample size because Rajala *et al.* (2016) collected more than 600 samples while in the current study sample size were restricted to 300 [22].

In the current study, the prevalence of brucellosis was recorded higher in goats as compared to sheep. Lower prevalence of brucellosis in sheep as compare to goats was stated by Gul *et al.* (2014) by using RBPT and ELISA. Specie wise prevalence of brucellosis in the current study is in accordance with the findings of various researchers [23, 24, 25, 26]. Higher prevalence of brucellosis in goats might be due to the fact that farmers in the study area kept goats and cattle in the same place while sheep are mostly reared separately. This statement is endorsed by the findings of Likov *et al.* (2010) farming of bovine and caprines all together increases the chances of the prevalence of brucellosis in goats.

Gender wise prevalence of brucellosis in the current study was more in female as compare to male. Higher prevalence of brucellosis in the female is also recorded by Din *et al.* (2013) in Bhimber, Azad Kashmir, Akbarmehr and Ghiyamirad (2011) in Iran, Rahman *et al.* (2011) in Bangladesh [27, 28, 29]. Possible reason for this higher prevalence might be due to the reason that female reproductive tract is a potential reservoir for the bacteria and can propagate easily and cause disease. This statement is in line with the findings of Gul *et al.* [30]. Mal husbandry practices like keeping animals over-crowded, no differentiation between aborted and pregnant animals rearing and to use brucellosis affected/cARRIER male animals for breeding purpose lead in a higher prevalence of brucellosis in animals.

5. Conclusion

From the present study it is concluded that Brucellosis prevalence in caprines and ovines through RBPT (VRI), RBPT (Lillidale UK), ELISA and PCR was 4.33%, 3.67%, 3.67% and 3.33% respectively. The brucellosis is prevailing day by day. All the techniques showed insignificantly different results. This study revealed that all techniques are reliable, sensitive and specific to detect brucellosis in small ruminants. PCR is more specific technique than RBPT and ELISA, and is confirmatory test to detect brucellosis in small ruminants, but need expertise and accuracy to perform. Goats showed insignificantly higher prevalence percentage than sheep population, among that female population of both goats and sheep showed insignificantly higher prevalence

percentage of brucellosis. This study revealed that brucellosis is an equally distributed in goats and sheep of Peshawar. Male population is as much on stake of brucellosis as of female population of small ruminants, because farmers raise male and female sheep/goats together. They carry infections and easily transmit it to herd mates. Serological techniques (RBPT and ELISA) are sensitive techniques to detect brucellosis in small ruminants but may lead to false positive or false negative results, both techniques use weak antigens of *B. melitensis* and *B. abortus*.

6. Acknowledgment

The contribution of all the authors is highly acknowledged.

7. References

- Gorvel JP, Moreno E. Brucella intracellular life: from invasion to intracellular replication. *Veterinary Microbiology*. 2002; 90:281-97.
- Abubakar M, Mansoor M, Arshed MJ. Bovine brucellosis: old and new concepts with Pakistan perspective. *Pakistan Veterinary Journal*. 2010; 32:147-155.
- Lopes LB, Nicolino R, Haddad JPA. Brucellosis - risk factors and prevalence: a review. *The Open Veterinary Science Journal*. 2010; 4:72-84.
- Lilenbaum W, de Souza GN, Ristow P, Moreira MC, Frágua S, Cardoso VS et al. A serological study on *Brucella abortus*, caprine arthritis-encephalitis virus and Leptospira in dairy goats in Rio de Janeiro, Brazil. *Veterinary Journal*. 2007; 173:408-412.
- Seleem MN, Boyle SM, Sriranganathan N. Brucellosis: A reemerging zoonosis. *Veterinary Microbiology*. 2010; 140:392-398.
- Radostits OM, Gay CC, Blood DC, Hinchcliff KW. *Veterinary Medicine*, 9th Ed, ELBS Bailliere Tindall, London, 2000, 870-871.
- Behzadi MA, Mogheiseh A. Outbreak investigation of brucellosis at a kennel in Iran. *Pakistan Veterinary Journal*. 2011; 31:379-380.
- Maurin M, Maurin M. Brucellosis at the dawn of the 21st century. *Médecine et Maladies Infectieuses*. 2005; 35:6-16.
- Abubakar M, Mansoor M, Arshed MJ. Bovine brucellosis: old and new concepts with Pakistan perspective. *Pakistan Veterinary Journal*. 2010; 32:147-155.
- Abubakar M, Mansoor M, Arshed MJ. Bovine brucellosis: old and new concepts with Pakistan perspective. *Pakistan Veterinary Journal*. 2010; 32:147-155.
- Robinson A. Guidelines for coordinated human and animal brucellosis surveillance. In: FAO animal production and health. 2003, 156.
- Glynn MK, Lynn TV. Brucellosis. *Journal of American Veterinary Association*. 2008; 233:900-908.
- Elfaki MG, Nakeeb A, Al-Rabiah SM. Evaluation of culture, tube agglutination and PCR methods for the diagnosis of brucellosis in humans. *International Medical Journal of Experimental and Clinical Research*. 2005; 11:65-74.
- Bailey GG, Krahn JB, Drasar BS, Stoker NG. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *Journal of Tropical Medicine Hygiene*. 1992; 95:271-5.
- Lopes LB, Nicolino R, Haddad JPA. Brucellosis - risk factors and prevalence: a review. *The Open Veterinary Science Journal*. 2010; 4:72-84.
- Shabbir MZ, Khalid RK, Freitas DM, Javed MT, Rabbani M, Yaqub T et al. Serological evidence of selected abortifacients in a dairy herd with history of abortion. *Pakistan Veterinary Journal*. 2013; 33:19-22.
- Hussain MA, Nazeer S, Rajput IR, Khan IZ, Mujeeb-ur-Rehman, Hayat S. Study on seroprevalence of brucellosis in caprine in Kohat Khyber Pakhtunkhwa. *Lasbela Uni J Sci Tech*. 2014; 3:14-20.
- Ali S, Ali Q, Neubauer H, Melzer F, Elschner M, Khan I et al. Seroprevalence and risk factors associated with brucellosis as a professional hazard in Pakistan. *Foodborne Pathog Dis*. 2013; 10:500-505.
- Radostits OM, Gay CC, Blood DC, Hinchcliff KW. *Veterinary Medicine*, 9th Ed, ELBS Bailliere Tindall, London, 2000, 870-871.
- Ali S, Akhter S, Neubauer H, Melzer F, Khan I, Ali Q et al. Ovine and Caprine brucellosis in Pakistan. *The Journal of Infection in Developing Countries*. 2015; 9(5):470-475.
- Rajala EL, Grahn C, Ljung I, Sattarov N, Boqvist S, Magnusson U. Prevalence and risk factors for *Brucella* seropositivity among sheep and goats in a peri-urban region of Tajikistan. *Trop Anim Health Prod*. 2016; 48:553-556.
- Likov B, Nenova-Poliakova R, Tomova I, Kamenov P, Boikovski I, Rubenova H et al. Epidemiological characteristics of brucellosis in Sheep and Goats in Bulgaria: 2005-2008. *Secondary Biological Medical Science*. 2010; 1:55-64.
- Akbarmehr J, Ghiyamirad M. Serological survey of brucellosis in livestock animals in sarab city (East Azarbayjan province) Iran. *African Journal of Microbiology Research*. 2011; 5:1220-1223.
- Rahman MS, Faruk MO, Her M, Kim JY, Kang SI, Jung SC. Prevalence of brucellosis in ruminants in Bangladesh. *Veterinarni Medicinia*. 2011; 56:379-385.
- Bonföh B, Kasymbekor J, Joktobaer N, Marcus G, Schheth DT, Zinsstag J et al. Representative seroprevalence of brucellosis in humans and livestock in Kyrgyzstan. *Ecological Health*. 2012; 9:132-138.
- Ali S, Akhter S, Neubauer H, Melzer F, Khan I, Ali Q et al. Ovine and Caprine brucellosis in Pakistan. *The Journal of Infection in Developing Countries*. 2015; 9(5):470-475.
- Din AMU, Khan SA, Ahmad I, Rind R, Hussain T, Shahid M et al. A study on the seroprevalence of brucellosis in human and goat populations of district Bhimber, Azad Jammu and Kashmir. *Journal of Animal and Plant Science*. 2013; 23:113-118.
- Akbarmehr J, Ghiyamirad M. Serological survey of brucellosis in livestock animals in sarab city (East Azarbayjan province) Iran. *African Journal of Microbiology Research*. 2011; 5:1220-1223.
- Rahman MS, Faruk MO, Her M, Kim JY, Kang SI, Jung SC. Prevalence of brucellosis in ruminants in Bangladesh. *Veterinarni Medicinia*. 2011; 56:379-385.
- Gul ST, Khan A, Rizvi F, Hussain I. Sero-prevalence of brucellosis in food animals in the Punjab Pakistan. *Pakistan Veterinary Journal*. 2014; 34:454-458.