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The Prevalence of *Brucella Melitensis* among health worker, butchers and livestock holders in district Abbottabad, Khyber Pakhtunkhwa, Pakistan

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Abstract
Brucellosis is a prolonged contagious bacterial sickness affecting human beings and several sort of native and wild animals. The aim of this study was to find out the prevalence of brucellosis among health care person in district Abbottabad. The current study was conducted at Veterinary Research and Disease Investigation Centre Abbottabad. During this study 200 samples were run in different times of fractions. The data about risk factors linked with brucellosis was collected on a pre-designed questionnaire. Initially all the collected samples were diagnosed for antibodies against *brucella* by serum plate agglutination test and rose bangle plate test. The Seropositive samples were confirmed by Enzyme linked immunosorbent Assay (ELISA). RBPT and SPAT being screening test proved maximum number of positive cases which were when tried through ELISA showed some negative results. Out of 200 cases the positive case were 52 through RBPT and 50 through SPAT. The ELISA eventually when conducted out of 200 samples only 18 were positive. This shows high specificity of ELISA. In terms of overall seroprevalence percentage the present study depicted that brucellosis was 26% through RBPT and 25% through SPAT and 9% through ELISA. Most of the cases which were positive grouped in the age between 21 to 40 years. Professional and occupational workers are more prone to the infection in case of the present study; mostly positive cases were occupational workers and professional individuals.
Key words: Prevalence; *Brucella Melitensis*; Health worker; Butchers; livestock holders

Introduction

Brucellosis is a prolonged contagious bacterial sickness affecting human beings and several sort of native and wild animals. Brucellosis in human is termed as Bang's disease, Maltese fever, Mediterranean fever, or Undulant fever (Dahouk *et al*., 2005). Human brucellosis has symptoms from mild ones like flue, and may be as complicated as damaging musculoskeletal system, the Heart, and Nervous system (Galińska, 2013). The illness is elevated from the countries with less adaptation to standards to protect animals and that of general population health. States belonging to central and south America, Africa, the Caribbean, Mediterranean sea Basin (North Africa, Turkey, Spain, Portugal, Italy, South France, Greece), and Asia are having elevated level of getting the infection. The causative agent of brucellosis is an aerobic, gram negative rod zoonotic bacteria. (Dziubek *et al*., 2012). The different species of *brucella* causes disease in variety of living organisms that included pets, breading ones, and small rodents, marine animals and birds. Cattle, dog, sheep, poultry (breading stock), and rodents and hares (wild animals) stand vector and container of the illness in beings. Recently, *B.canis, B.melitensis, B.abortus B.marina*, and *B.suis,* are recognized as human pathogens. Either elevated cases due to *B.melitensis* as the top dangerous species, than *B.suis* and *B.abortus* respectively (Halling *et al*., 2005). Swine, sheep, goats, and other pets are the sources of human beings to get infection. Also foxes, roe deer, wild rabbits, hares (wild animals), shepherd dogs especially are the pools and diffuser of the germ. Highly exposed beings are Vet doctors, and assistant, technicians, zootechnicians, employee of meat processing enterprises, the fodder processing company, insemination service employees, and farmer working on multi-herd farms (e.g cattle man, private farmer).

Globally it is a great issue as it has the ability to destruct. The cost produce bio-weapon is less and can be sprayed using normal aerosol spraying machines. Very little of pathogens are having the potential to become colonized. If intervention activities are lacking, bioterrorism attack is done. The factors exposing beings to be infected are, Integration of contaminated products like unpasteurized milk and products, (including camel, goat, cow milk), and red meat, Supervision of infected animals, Handling of cultures of brucella species in laboratories, Dairy workers, Slaughterhouse workers, History of travel to endemic areas, Veterinarians, and Abattoir workers (Buchanan *et al*., 1980 and Corbel, 2007).

Pets are responsible mainly for human brucellosis (Smits and Kadri, 2005). Entrance can be achieved through broken skin, GIT (gastrointestinal tract), RT (respiratory tract), and conjunctiva (Bachanan *et al*., 1974). After entering the bacteria are engulfed by polymorphonulcear leukocytes that normally lost taken hold on them and moreover phagocytosed by macrophages (Lopez, 1989). Transporting by macrophages, the microbe moves to lymphoid tissues where they can survive in spleen, memory gland, liver, bone marrow, kidney and joints and form granulomatous lesions and abscesses, which complicates the disease (Ko and Splitter, 2003). While in macrophages, the bacteria prevent the lysosome-phagosome fusion (Gorvel and Moreno, 2002). There in acidic pH bacteria multiply in endoplasmic reticulum in special compartments while having the residue in cells integrity through type-IV secretion system (Boschiroli *et al.,* 2002). Subsequent to replication, the bacteria came out of the cell via induced cell necrosis and hemolysis (Gorvel and Moreno, 2002). And create frame to survive and replicate (Pappas *et al*., 2006), and allow the escape of microbe from extracellular immune mechanism (that is antibodies and the complement). Complete set of immune response that induces both innate and adoptive immune response are initiated (Golding *et al*., 2001). Cytokines, Interleukine1, 12, Interferon-gamma and Tumor Necrosis Factor- Alpha are the ones that have the key role in pathogenesis of disease. Th1/Th2 is thought to have involvement in susceptibility and resistance (Galnakis *et al*., 2002 and Pasquali *et al*., 2001). Th1 mediates the needed pattern to effect and resist the organism present between the cells, similarly Th2 is important to fight this disease (Yingst and Hoover, 2003).

Brucellosis has a diverse epidemiology which fluctuates regularly. The immune system of an individual very broad horde and resistant surroundings help brucellosis to breed. In the area where there is endemic of Brucellosis, globally, the incidence of disease ranges from less than 0.01 to more than 200 out of one hundred thousand individuals (Boschiroli *et al*., 2001), with real occurrence is still unknown in states that induced Pakistan because of under reported and misdiagnosed cases, the actual occurrence may be twenty five times elevated to what is reported (Mantur *et al*., 2007). There is limited data in the literature about brucellosis in Pakistan. This study was therefore carried out to assess the prevalence of brucellosis among health care person in district Abbottabad.

Materials and methods

Study area

The current research study was conducted at Microbiology section of Veterinary Research and Disease Investigation Center Abbottabad.

Data collection

A questionnaire was designed to collect demographic and clinical information of the subject. Different health care centers were visited for filling questionnaire. A detail history of the individuals were collected with respect to their nature of work occupation, history of consumption of raw milk, fever history, joint pain, weakness and weight loss etc.

Sample collection

200 Blood samples were collected from groups of health workers of district Abbottabad, KPK, Pakistan

Samples processing

A 5ml sample collected from each individual. The serum was separated through centrifugation at 300 rpm for 15 minutes and used for serological tests such as ELISA (Enzyme Linked Immunosorbent Assay), RBPT (Rose Bengal Plate Test) and SPAT (Serum Plat Agglutination Test)

Statistical Analysis

The data obtained was tabulated in the Microsoft Excel spreadsheet and analyzed using the Statistical Package for Social Sciences (SPSS), version 21.0. A p value of less than 0.05 was taken as significant.

Results

In the current study, overall 200 samples of human blood were used to determine the Prevalence of *Brucella melitensis* among the Health workers, Livestock holders and Butchers in District Abbottabad. The blood samples were collected in the month of April 2018 to December 2018 and divided into various categories (Table 1).

Table 1: Categories of collected samples

Sub Category	Number of sample Collected
1. Health workers	
i) Vets	07
ii) Veterinary Assistants	29
iii) Laboratory workers	53
2. Livestock Holders	
Farm workers	46
Nomads	44
3.Butchers	21
Total	200

Prevalence of *B.Melitensis* in District Abbottabad

In the 3 main categories of health workers, livestock holders and butchers, prevalence of *B.melitensis* varied. Positive samples through RBPT were 52 (26%), positive samples through SPAT were 50 (25%), and positive samples through ELISA were 18 (9%) respectively. Among these professional groups the prevalence of *B.melitensis* was high in Health workers. In the first group, out of 89 samples, 27 were positive through RBPT, 23 were positive on SPAT, and 3 were positive on ELISA, the percentage was 58.4, 25.8, and 3.3 respectively. In the second group that was livestock holders, out of 90 samples 19 were positive on RBT, 25 were positive on SPAT, and 15 were positive on ELISA, the percentage was 21.1, 27.7 and 16.6 on RBPT, SPAT and ELISA correspondingly. In the third group, that was butchers, out of 21 collected samples, 6 was positive on RBPT, 2 was positive on SPAT, and 0 on ELISA, the percentage through RBPT, SPAT, and ELISA was 28.5, 9.5 and 0 respectively.

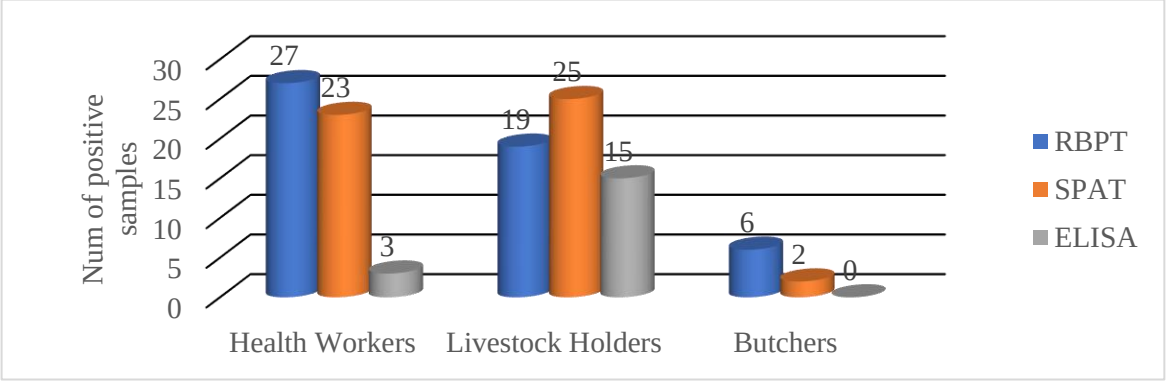

Fig 1: Showing positive samples of 3 main categories

Table 2: Sub category wise distribution of positive samples through RBPT, SPAT and ELISA

Category	Total samples	RBPT Positive	SPAT Positive	ELISA Positive
1.Health Workers a. Veterinary Doctors	7	3 (42.8%)	2 (28.5%)	1 (14.2%)
b. Veterinary Assistants	29	8 (27.5%)	6 (20.6%)	0 (0%)
c. Laboratory Workers	53	16 (30.1%)	15 (28.3%)	2 (3.7%)
2.Livestock Holders				

a. Farm Workers	46	7 (15.2%)	11 (23.9%)	10 (21.7%)
b. Nomads	44	12 (27.2%)	14 (31.8%)	5 (11.3%)
3. Butchers	21	6 (28.5%)	2 (9.5%)	0 (0%)

Table 3: Gender and age-group wise distribution of RBPT

Age-group			RBPT		Total	P value
			Positive	Negative		
0-20	Patient Gender	Male	4	18	22	.101
		Female	1	3	4	
	Total		5	21	26	
21-40	Patient Gender	Male	34	92	126	.015*
		Female	2	6	8	
	Total		36	98	134	
41-60	Patient Gender	Male	8	25	33	.806
		Female	2	1	3	
	Total		10	26	36	
61	Patient Gender	Male	1	3	4	0.001*
	Total		1	3	4	
Total	Patient Gender	Male	47	138	185	
		Female	5	10	15	
	Total		52	148	200	

Table 4: Gender and age-group wise distribution by SPAT

Age-group			SPAT		Total	P value
			Positive	Negative		
0-20	Patient Gender	Male	3	19	22	.891
		Female	4	0	4	
	Total		7	19	26	
21-40	Patient Gender	Male	27	99	126	0.012*
		Female	5	3	8	
	Total		32	102	134	
41-60	Patient Gender	Male	10	23	33	.982
		Female	1	2	3	
	Total		11	25	36	
61	Patient Gender	Male		4	4	0.001*
	Total			4	4	
Total	Patient Gender	Male	40	145	185	
		Female	10	5	15	
	Total		50	150	200	

Table 5: Age-group and gender wise distribution by ELISA in Table 5.c

Age-group			ELISA		Total	P value
			Positive	Negative		
0-20	Patient Gender	Male	1	21	22	0.189
		Female	0	4	4	
	Total		1	25	26	

21-40	Patient Gender	Male	11	115	126	0.038*
		Female	3	5	8	
	Total		14	120	134	
41-60	Patient Gender	Male	3	30	33	.298
		Female	0	3	3	
	Total		3	33	36	
61	Patient Gender	Male		4	4	0.001*
	Total			4	4	
Total	Patient Gender	Male	15	170	185	
		Female	3	12	15	
	Total		18	182	200	

P* value based on chi-square test (significant value)

Discussion

Globally, Brucellosis is a zoonotic illness. However, from many advanced states such as in Europe, Australia, Israel, Japan, Canada, and New Zealand it had exterminated (Geering *et al*., 1995), in regions of high endemicity such as the Middle East, Africa, Mediterranean, Latin America parts of Asia and, yet it remains an uncontrolled problem (Refai, 2002). Except cats, nearly all domestic species can be affected with brucellosis, which are resistant to *Brucella* infection. Consider the damage done by animal infections in terms of conditions of abortions, weak off springs, decreased milk production, infertility, lameness and weight loss, this is one of the utmost serious illnesses of domestic animals. For the trade it's also a major impediment. As a consequence of acute metritis death might occur, followed via retained fetal membranes (Raza *et al.,* 2014). Brucellosis may be professional, through bioterrorism, refreshments or travelling and may be related to tourisms. Agronomic, tourism, communal and public settings may be adjusted and novel brucellosis types may arise (Godfroid *et al*., 2015). It mostly upset slaughterers, veterinarians and slay workers there for it is termed as work related illness (occupational disease). It is normally transmitted by contact to substances that have skin-scratches or by infected lives (Silva *et al*., 2000).

This study concludes that brucellosis is a disease of public health importance, with a high seroprevalence among the health workers, livestock holders and butchers of district Abbottabad. It shows non-specific symptoms such as arthralgia, night sweats, fever, weight loss, myalgia and anorexia. Brucellosis is a multi-system illness with an inclination for chronicity. The infected individuals might develop gastrointestinal, hematologic, and skeletal difficulties, while rare cases may advance the further serious ones that are endocarditis, osteomyelitis, and meningitis. For protection from infection the workers should use personal protective devices and regarding modes of prevention from brucellosis an educational program should be launched for the professional workers. In addition, effective working guidelines for the professionals or occupational must be developed (Sari *et al*., 2008).

Out of 200 cases the positive case were 52 through RBPT and 50 through SPAT. The Elisa eventually when conducted out of 200 samples only 18 were positive. This shows high specificity of Elisa. RBPT and SPAT are the test which are used in the field for general screening of the samples which are later confirmed thorough serological testing for the accurate results. In terms of overall seroprevalence percentage the present study depicted that brucellosis was 26 % through RBPT and 25 % through SPAT and 9.5 % through ELISA. Most of the cases which were positive grouped in the age between 21 to 40 years (Nahar and Ahmed 2009).

Professional and occupational workers are more prone to the infection in case of the present study; mostly positive were occupational workers and professional individuals. Mukhtar, 2010, had similar finding in occupational workers of the slaughter house had the highest percentage that was 21.7%.

The samples which were collected from the female workers were reluctant to show the history due to the traditional impediments and may be due some other reasons well known to them. Since the brucellosis severely affects the pregnant females and may cause the sudden abortion. The positive cases if allowed to

take the history might have come with such results. The similar findings have also been shown by Manat *et al*., 2016.

The butchers although at the risk, yet showed only 4-6 samples positive, may be due to the immune system of the individuals whose samples were taken most of them were below 30 years of age. This shows the time of high efficacy of the immune system. The *B.melitensis* may enter through the skin abrasions but eradicated by the specific or the adoptive immunity. TH1 and TH2 may play the role in order to resist the *B.melitensis*, the same was identified by Yingst and Hoover, 2003.

This study shown the positivity whole the livestock holders, laboratory workers, Farm workers, veterinarians, slaughterhouse workers, dairy workers, this clearly depicts that *B.melitensis* spreads from animal handling, through the handling of cultures in laboratory while experimentation the study conducted by Buchanan *et al*., 1974 and Cerbel *et al*., 2007 has very clearly conducted the affectties which shown the holders in any case are at risk. Some of the positive cases even had no signs of brucellosis and they were of the view that they have been involved in such agony on the other hand some showed signs of the disease fever etc.

The positive cases when further examined in details, it was clear that the environmental factors like personal hygiene and the community hygiene has played an active role this connection. In addition to the animal husbandry practices, milk handling, food habits has also played a vital role in the transmission of Brucella, the similar findings and results were also covered by Romani *et al*., 1996.

A number of positive cases through SPAT and RBPT also revealed that pets also contribute in the transmission and development of Brucella. When the positive cases were looked in the details 15-20% of the cases showed the pets owners like dogs. The literature reviewer also indicated that the pets also responsible for human Brucella (Henk *et al*., 2005) the blood samples from the positive cases when examined showed leukocytes including lymphocytes like CD4 and CD8. Ottones *et al*., 2000 when examined the blood they came up with almost the same findings. The immunoglobulin test when conducted the titration, it showed in few cases high titer. This showed high immune response against Brucella (Pappas *et al*., 2005). When tested the blood against Brucella they found that these antibodies were IgM and IgG depending upon the immune system.

Some of the positive cases including health workers and butchers were showed the clinical signs of the disease, this may be due to unavailability of the proper diagnostic techniques in the interest of suffered sufferers some cases may wrongly take brucellosis as any other illness and might have used different drugs to combat the signs of the disease. The drugs may include antibiotics, analgesics, and antipyretics and in few cases anti-fungal for the skin infections as well. This is important for the education of masses that the drugs may be used only after comprehensive examination and confirmatory diagnosis. The sufferers in may study doing their manual in routine activities without knowing that they are the carriers of brucella. Bertu *et al*., 2010 have also highlighted different treatment strategies and has shown few drugs specific for the subsiding of the infection.

In farmers, the prevalence was 23.9% in comparison to other employers. These findings are in in accordance with the previous study who reported 33% *Brucella* in farmers (Shahid *et al*., 2014). This might be due to the fact that farmers and rural residents have close connection with animals, which are the main reservoir of brucellosis.

Conclusion

Our study concludes that *Brucella* is zoonotic and spreads from animal to health workers in general and veterinarians and livestock holders in specific. The prevalence of *B.melitensis* in District Abbottabad is 26% through RBPT, 25% through SPAT and 9% through ELISA. The prevalence of *B.melitensis* in health workers is 58.4% among the main three professional groups. Butchers are at the risk so far as the transmission of the *B. melitensis* is concerned but this study shows that only 4-6 samples were positive only by RBPT. Our study recommended that government should arrange awareness programs in general population about brucellosis and their associated risk factors. Unpasteurized milk and other product should not be consumed to control this disease.

References

1. Al Dahouk, S., Nöckler, K., Hensel, A., Tomaso, H., Scholz, H. C., Hagen, R. M., & Neubauer, H. (2005). Human brucellosis in a nonendemic country: a report from Germany, 2002 and 2003. *European Journal of Clinical Microbiology and Infectious Diseases*, *24*(7), 450-456.
2. Bertu, W. J., Ajogi, I., Bale, J. O. O., Kwaga, J. K. P., &Ocholi, R. A. (2010). Sero-epidemiology of brucellosis in small ruminants in Plateau State, Nigeria. *African Journal of Microbiology Research*, *4*(19), 1935-1938.
3. Boschiroli, M. L., Foulongne, V., & O'Callaghan, D. (2001). Brucellosis: a worldwide zoonosis. *Current Opinion In Microbiology*, *4*(1), 58-64.
4. Boschiroli, M. L., Ouahrani-Bettache, S., Foulongne, V., Michaux-Charachon, S., Bourg, G., Allardet-Servent, A., ... & O'Callaghan, D. (2002). Type IV secretion and Brucella virulence. *Veterinary Microbiology*, *90*(1-4), 341-348.
5. Buchanan, T. M., & Faber, L. C. (1980). 2-mercaptoethanol Brucella agglutination test: usefulness for predicting recovery from brucellosis. *Journal Of Clinical Microbiology*, *11*(6), 691-693.
6. Corbel, M. J., Ariza, J., Bosilkovski, M., Cascio, A., Colmenero, J. D., Falagas, M. E., ... & Solera, J. (2007). Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. *Plos Medicine*, *4*(12), e317.
7. Corbel, M. J., Ariza, J., Bosilkovski, M., Cascio, A., Colmenero, J. D., Falagas, M. E., ... & Solera, J. (2007). Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. *Plos Medicine*, *4*(12), e317.
8. Dziubek, A. M. (2012). Some of the problems concomitant with the removal of nitrogen compounds in Poland. *Environment Protection Engineering*, *38*(4), 163-169.
9. Galanakis, E., Makis, A., Bourantas, K. L., &Papadopoulou, Z. L. (2002). Interleukin-3 and interleukin-4 in *Childhood Brucellosis. Infection*, *30*(1), 33-34.
10. Galinska, E. M., &Zagórski, J. (2013). Brucellosis in humans-etiology, diagnostics, clinical forms. *Annals Of Agricultural And Environmental Medicine*, *20*(2).
11. Geering, W. A., Forman, A. J., & Nunn, M. J. (1995). *Exotic Diseases of Animals: A Field Guide for Australian Veterinarians*. Australian Government Publishing Service. 81(3-5), 301-306.
12. Godfroid, J., Cloeckaert, A., Liautard, J. P., Kohler, S., Fretin, D., Walravens, K., ... &Letesson, J. J. (2015). From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Veterinary Research*, *36*(3), 313-326.
13. Golding, B., Scott, D. E., Scharf, O., Huang, L. Y., Zaitseva, M., Lapham, C., ... & Golding, H. (2001). Immunity and protection against Brucella abortus. *Microbes and Infection*, *3*(1), 43-48.
14. Gorvel, J. P., & Moreno, E. (2002). Brucella intracellular life: from invasion to intracellular replication. *Veterinary microbiology*, *90*(1-4), 281-297.
15. Halling, S. M., Peterson-Burch, B. D., Bricker, B. J., Zuerner, R. L., Qing, Z., Li, L. L., ... & Olsen, S. C. (2005). Completion of the genome sequence of Brucella abortus and comparison to the highly similar genomes of Brucella melitensis and Brucella suis. *Journal of Bacteriology*, *187*(8), 2715-2726.
16. Henk, L., Smits. And Kadri, S. M. (2005). Brucellosis in India: deceptive infectious disease, *Indian Journal of Medicine and Res*, (122) 375-384.
17. Ko, J., & Splitter, G. A. (2003). Molecular host-pathogen interaction in brucellosis: current understanding and future approaches to vaccine development for mice and humans. *Clinical Microbiology Reviews*, *16*(1), 65-78.
18. Lopez-Merimo, A., & Lopez-Santiago, R. (1989). Immunology of brucellosis in humans. In Brucellosis (pp. 45-58). *Butterworths London*.
19. Manat, T. D., Chaudhary, S. S., Singh, V. K., Patel, S. B., & Puri, G. (2016). Hematobiochemical profile in Surti goats during post-partum period. *Veterinary world*, *9*(1), 19.
20. Mantur, B. G., Amarnath, S. K., & Shinde, R. S. (2007). Review of clinical and laboratory features of human brucellosis. *Indian Journal Of Medical Microbiology*, *25*(3), 188.
21. Mukhtar, F. (2010). Brucellosis in a high risk occupational group: seroprevalence and analysis of risk factors. *JPMA-Journal of the Pakistan Medical Association*, *60*(12), 1031.
22. Nahar, A., & Ahmed, M. U. (2009). Sero-prevalence study of brucellosis in cattle and contact human in Mymensingh district. *Bangladesh Journal of Veterinary Medicine*, *7*(1), 269-274.

23. Ottones, F., Dornand, J., Naroeni, A., Liautard, J. P., &Favero, J. (2000). Vγ9Vδ2 T cells impair intracellular multiplication of Brucella suis in autologous monocytes through soluble factor release and contact-dependent cytotoxic effect. *The Journal of Immunology*, *165*(12), 7133-7139.
24. Pappas G ., Belo, A. C. (2005). Pappas G, Akritidis N, Bosilkovski M, Tsianos E. *N Engl J Med*, *352*, 2325-36.
25. Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., &Tsianos, E. V. (2006). The new global map of human brucellosis. *The Lancet Infectious Diseases*, *6*(2), 91-99.
26. Pasquali, P., Adone, R., Gasbarre, L. C., Pistoia, C., &Ciuchini, F. (2001). Mouse Cytokine Profiles Associated withBrucella abortus RB51 Vaccination or B. abortus2308 Infection. *Infection And Immunity*, *69*(10), 6541-6544.
27. Raza, S., Khan, N., & Amin, Y. (2014). Epidemiological study of brucellosis in equines of district Peshawar Khyber Pakhtunkhwa Pakistan. *Int. J. Curr. Microbiol. App. Sci*, *3*(2), 795-800.
28. Refai, M. (2002). Incidence and control of brucellosis in the Near East region. *Veterinary Microbiology*, *90*(1-4), 81-110.
29. Romani, N., Reider, D., Heuer, M., Ebner, S., Kämpgen, E., Eibl, B., ... & Schuler, G. (1996). Generation of mature dendritic cells from human blood An improved method with special regard to clinical applicability. *Journal of immunological methods*, *196*(2), 137-151.
30. Shahid, S., Mahmood, N., Chaudhry, M. N., Sheikh, S., & Ahmad, N. (2014). Assessment of impacts of hematological parameters of chronic ionizing radiation exposed workers in hospitals. *FUUAST Journal of Biology*, *4*(2), 135.
31. Silva, I., Dangolla, A., &Kulachelvy, K. (2000). Seroepidemiology of Brucella abortus infection in bovids in Sri Lanka. *Preventive Veterinary Medicine*, *46*(1), 51-59.
32. Smits, H. L., & Kadri, S. M. (2005). Brucellosis in India: a deceptive infectious disease. *Indian Journal of Medical Research*, *122*(5), 375.
33. Yingst, S., & Hoover, D. L. (2003). T cell immunity to brucellosis. *Critical Reviews In Microbiology*, *29*(4), 313-331.