

Study of Correlation between Some Serological Parameters with Brucella Militance Infection

Amidah Ali Atiyah*, Asmaa Essa Mahmood, Sahar Abd Al-Wahhab, Nael Mustafa

Department of Pathological Analysis, College of Applied Sciences, University of Samarra, Samarra, Iraq
Email: ami.iq30@yahoo.com

ABSTRACT

The current study aimed to measure the level of IgG, IgA, IgM antibodies, and complement factors C3, C4, in people infected with Brucella.militance in Samarra district, where 50 samples were collected from people with symptoms of B.militance infection and the result showed that only 15 samples By 30%, the remaining 35 samples were due to other causes, and by 70%. The results showed a significant increase in the level of immune proteins, with a concentration of (81.7753251) (30.0758857, respectively).

Keywords: (*B.militance*, IgG, IgM, IgA, (Radial Immuno Diffusion)

Correspondence:

Amidah Ali Atiyah
Department of Pathological Analysis, College of Applied Sciences, University of Samarra, Samarra, Iraq
Email: ami.iq30@yahoo.com

INTRODUCTION

Brucella bacteria cause brucellosis, which is one of the most common infectious animal diseases. The disease spreads at a high rate. There has been a significant increase over recent years in the incidence of this disease among the same animal group or between the two sexes due to poor management and limited resources, especially in developing countries. That brucellosis was identified in the late Roman era and was so named because of its similarity to the organism Brucellae, and later its name was modified Brucella, which is a derivative of cheese. Brucellosis stands in the first place in the list of bacterial diseases of animal origin, which is a common disease Between animals and humans Zoonoses arise in animals and are transmitted to humans directly or indirectly, and annually 500,000 cases are reported in endemic areas [1] [, [2]]. Nevertheless, this disease is a widespread infection in the Middle East and North Africa. However, the means of detecting its transmission are more than 100 years old, and the disease is still a problem all over the world, especially in developing countries, [3] [However, the World Health Organization (FAO / OIE / WHO) reported in 1997 that countries such as North America, North and Central Europe, Canada, and Japan, Australia, New Zealand and Southeast Asia are free of the disease. In addition, the disease is endemic in the Mediterranean region, central and eastern Latin America and parts of Asia [4]. Brucellosis in humans causes chills, cough, shortness of breath, high fever, emaciation, insomnia, headache, fatigue, arthritis, loss of appetite and weight, onset of septicemia, Mongolian fever, and endocarditis and may cause death, and intrauterine infections with the death of the fetus [5], [6]], It causes neurobrucellosis, meningoencephalitis, stroke, myelitis, peripheral neuropathy, and neuropsychiatric characteristics [7], [8]. And sensorineural deafness reported for spastic cerebral palsy [9]. And foreign skin pain in patients with latent empyema, [11] [10]. The current study aimed at: - (Diagnosing *B. militance* in people with brucellosis), measuring the concentration of IgA, IgM, and IgG antibodies, as well as measuring the concentration of complement proteins C4, C3 in infected people and using the immunodiffusion technique to detect *B. militance* Causing infection.

WORKING METHODS

Specimens collection: The study included 50 samples collected from external laboratories in the district of

Samarra / Salah El-Din, of both sexes, with an age group ranging between 8 - 13 years, where the people were suffering from symptoms of infection with *B. militance* and the results of the traditional examination showed Rose Bengal, which proved the presence of the objects In addition to the symptoms associated with the disease, it was confirmed that only 10 samples were due to *B. militance*, with a percentage of 20%, while the remaining 40 samples were due to other causes, and by 80%, control samples consisting of 5 blood samples from children of the age group were selected It is the same for the group of patients, and their safety has been confirmed by conducting the same examination for them. Working **Method of Rose bengalle. kit**

Samples were placed at room temperature and 1 40 was taken from each sample and placed on a glass slide and 2 drops of Rose bengalle reagents were added to it, according to the manufacturer's instructions.

How does Immunodiffusion radiale kit work

The Radial Immuno Diffusion (RID) or what is called the Mancini method was used, and the principle of work was based on showing a quantitative relationship between the antigen present in the acarose and the antibodies present in the serum, and the test was performed according to the manufacturer's instructions as titration dishes were prepared for each of IgG and IgM IgA, C3, C4 from LTA srl company, after which I opened the cap and left it at laboratory temperature for 5 minutes, then 5 microliters of serum of patients and healthy subjects were added to it in the pits designated for it in the gel using a fine pipette and the dishes were left for 72 hours at a temperature Laboratory without moving, and after that the sedimentation rings formed around the drilling as a result of the interaction of the specific antigen with its antigen in the gel were measured using a special lens to measure immunological analyzes. The concentration value in mg / dl was extracted by comparing the diameter of the sedimentation ring with the values fixed in the attached table with the dishes prepared by the manufacturer.

The diagnosis

The use of serological diagnostics by detection of antibodies (Wright reaction), antigens and antibody is considered as one of the confirmatory methods in diagnosis by means of IgM and IgG immunoglobulins [12].

RESULTS

The results of the current study showed a high concentration of antibodies to the positive samples for the Rose Bengal type IgA test, as the average concentration was 218.9 in the patient group compared to the control group 126.78. The patient group also recorded the highest deviation, which was 164.0925062 compared to the control group 117.4960768, and IgM recorded the highest average concentration in the patient group. When it was 184.53 compared to the control group 93.96, IgG recorded the highest mean concentration in the patient group, which was 1654.54 compared to the control group 1155.6. As in the table. 1 and 2. And scheme 1, and this result was similar to his findings [13]. As well as match what he arrived at [14]. This is due to the occurrence of a specific immune response represented by the production of antibodies. When comparing the results of the average antibody concentrations of a group of patients, it was found that the concentration of IgG increased by 1654.54 and the mean concentration of IgA reached 218.9, and the results of a study of the control group showed an increase in the concentration of the antibody type IgG. The mean

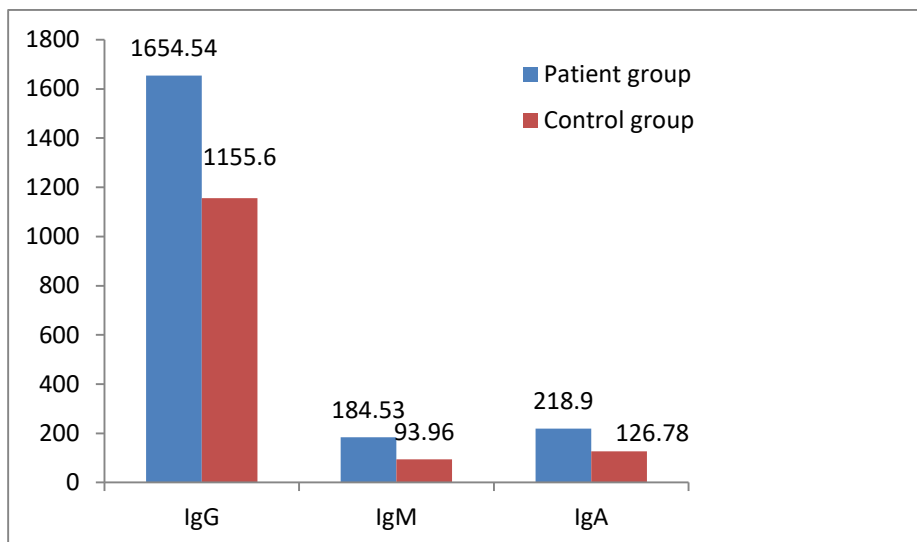
concentration of 1155.6 compared to the lowest concentration of IgM was 93.96. As in Table 1 and Scheme 3, the diameter of IgA spread in the patient group differed, with the largest diameter being 9 and the lowest diameter 4.2. Compared to the control group, the largest diameter was 7 and the lowest diameter was 4. As shown in Table 1. It was also observed that the diameter of the spread of the immune body IgM differed, with the largest diameter being 8.2 and the lowest diameter 5 compared to the control group, with the largest diameter being 7 and the lowest diameter 4.2. As shown in Table 2. The IgG immunodeficiency body's diameter also differed, with the largest diameter being 10.2 and the lowest diameter 5.8 compared to the control group, with the largest diameter being 9.7 and the lowest diameter 4.2. 10.2 in IgG and the lowest prevalence diameter was 4.2 in IgA. As per diagram. 2. The diameter of the spread of antibodies to the control group also differed, so the largest diameter appeared in IgG, 9.7, and the lowest diameter was 4 in IgA. Thus, the patient and control group were similar in that the diameter of the largest spread of the immune body IgG and the lowest diameter appeared at the immune body IgG as in Table 1 and 2.

Table1. The antibody concentration shows variation for the control group

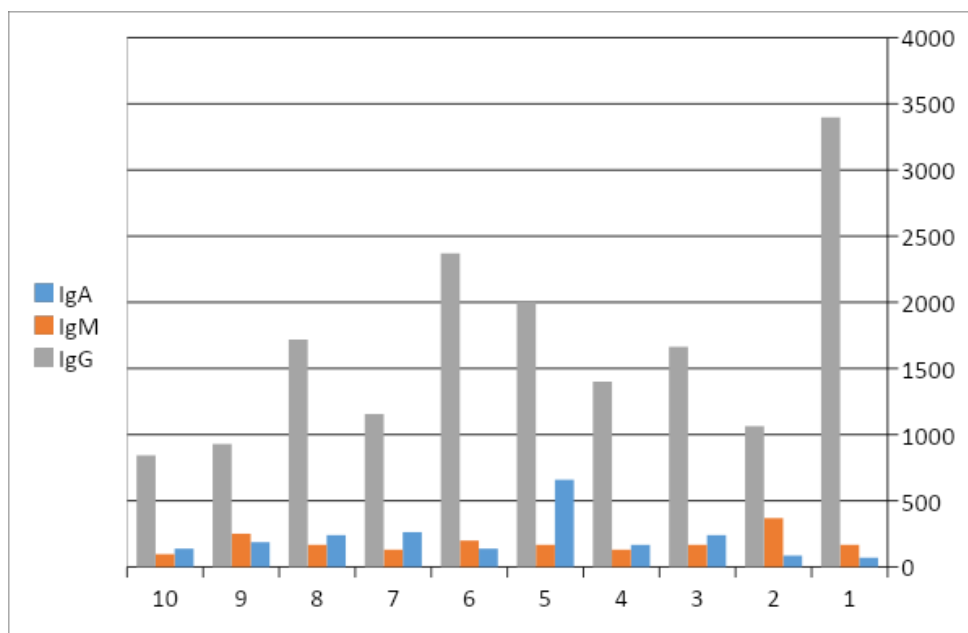
N	IgG		IgM		IgA	
	concentration	diameter	concentration	diameter	concentration	diameter
1	357.3	4.5	54.6	4.3	54.4	4
2	262.6	4.2	49.1	4.2	69.7	4.2
3	720.2	5.5	49.1	4.2	54.4	4
4	3037.2	9.7	65.9	4.5	94	4.5
5	1400.7	7	251.1	7	361.4	7
Average	1155.6		93.96		126.78	
Standard deviation	522.7		79.68855348		117.4960768	

Table 2. The antibody concentration shows the variation in the patient group

N	IgG		IgM		IgA	
	diameter	concentration	diameter	concentration	diameter	concentration
1	10.2	3398.3	6	167.3	4.2	69.7
2	6.3	1062.8	8.2	368.6	4.4	85.7
3	7.5	1663.9	6	167.3	6	240.5
4	7	1400.7	5.5	130.3	5.3	166.9
5	8.1	2003.6	6	167.3	9	659.1
6	8.7	2369.4	6.4	199.3	5	138.2
7	6.5	1155.8	5.5	130.3	6.2	263.2
8	7.6	1718.7	6	167.3	6	240.5
9	6	928.9	7	251.1	5.5	187
10	5.8	843.3	5	96.5	5	138.2
Average		1654.54		184.53		218.9
Standard deviation		817.97929		87.19800115		164.0925062



Scheme 1. Demonstrates the difference in the mean antibody concentration of the control group and the patients.



Scheme 2. Demonstrates the difference in antibody concentration for the patient group

The results of the current study also showed a decrease in the average C3 concentration in the patient group, as it reached 155.7 compared to the control group 158.25, while the average C4 concentration in the patient group increased by 41.81 compared to the control group 37.62, Tables 3 and 4 and Schemes 4 and 5. The highest deviation was found in C3 by 81.7753251 compared to C4 by 30.0758857, while the amount of deviation of C3 was 45.59 compared to C4 by 14.734 in the control group. As shown in Figure 5, the results showed a difference in the diameter, the prevalence of type C3 immune proteins, where the largest diameter was 11.5, the lowest diameter was 5.3, the largest diameter of C4 was 9.7 and the lowest diameter was 4 for the patient group, as shown in Table 4. The diffusion diameter of C3 and C4 immunoproteins of the control group also differed, with the largest diameter of 8 and 10 appearing for C3 and C4

respectively, and the lowest diameter reaching 4.2 in C3 and C4. As in a schedule. 5 and 6. The complement system consists of more than thirty proteins, some of which are free, and the other is bound to membranes that have the ability to interact with each other and with their stimulants, and the liver is the main source for the manufacture of complement proteins [15]. It represents one of the largest defense systems in the body as it plays an important role in controlling inflammatory reactions and getting rid of immune complexes, as well as activating cells to produce antibodies and defend against microorganisms [16]. Through the results, it was found that there is an increase in the level of C4 and C3 concentration in patients' serum with *B. Militance* compared with the control group, and these results are consistent with what they have reached [13]. The reason for this may be due to the role that C3 plays in the

alternative pathway of the complement in healing, but this differs with [17,18]. Which confirmed that human serum has a fatal effect on microorganisms and this effect appears depending on the classical complement pathway of the traditional complement. The component C3b has a short life in the liquid phase, that is, in the blood unless a suitable membrane is found on which the bacteriophage is fixed, and this membrane is present on it. And viruses and parasites, the absence of the membrane leads to its binding to the surface of red cells in the blood through the C3b receptors on the surface of erythrocytes called CR1 in the blood, but the autologous cells, including the

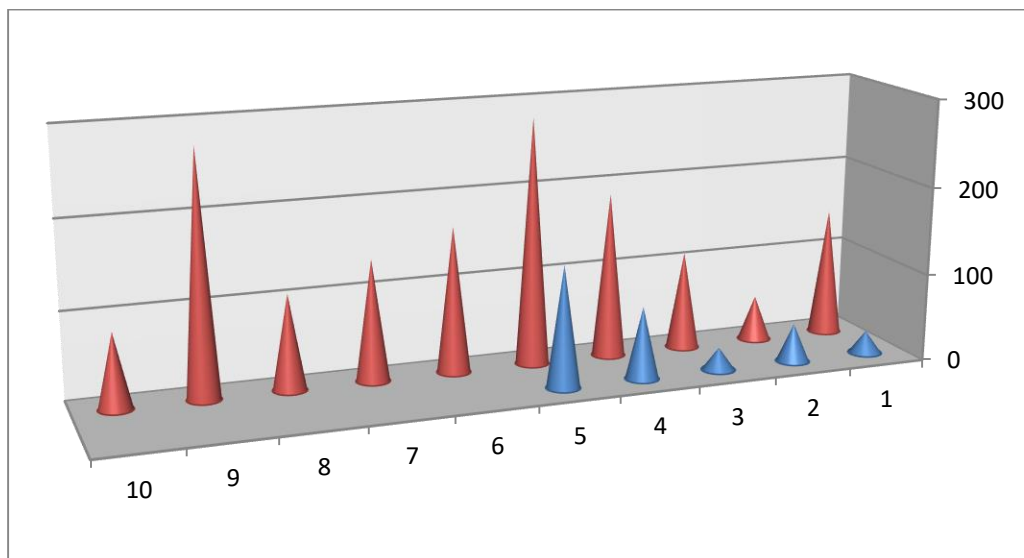
erythrocytes, are able to avoid the degradation by the complement by means of inhibiting factors C3-convertase. (C3- defines C4b2a and C2b) C2a and C4b form a complex i.e. the activation component or the transfer switch where C2a acts in it to make an enzyme to split or divide the other components, while C4b is subtracted to the small outer medium within the reaction zone. The presence of magnesium ions bisects the C3 convertase-C3 into C3a and C3b, then a C4b2a3b complex known as the C5-convertase that now splits, C3b is made up of C5 to C5a and C5b, the emergence of the enzyme convertase-5a [C5b].

Table 3. The concentration and diameter of C3 and C4 shows the variation for the control group.

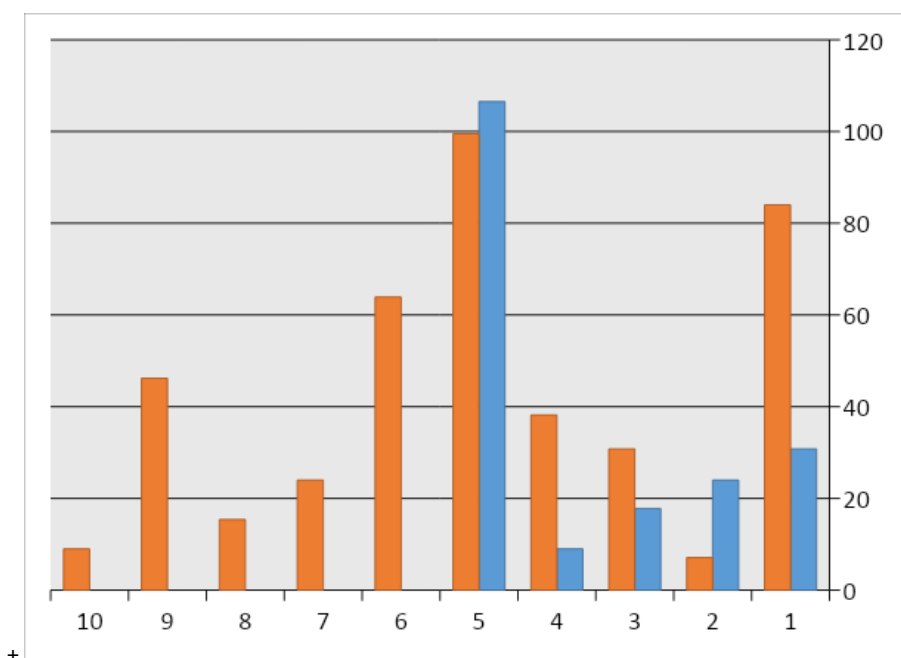
N	C ₃		C ₄	
	diameter	concentration	diameter	concentration
1	4.2	25.9	6	30.8
2	5	43.8	5.5	24
3	4.2	25.9	5	17.8
4	6.4	82.5	4.2	9
5	8	138.4	10	106.5
Average		158.25		37.62
Standard deviation		45.59		14.734

Table 4. The concentration and diameter of C3 and C4 show the variation for a group of patients.

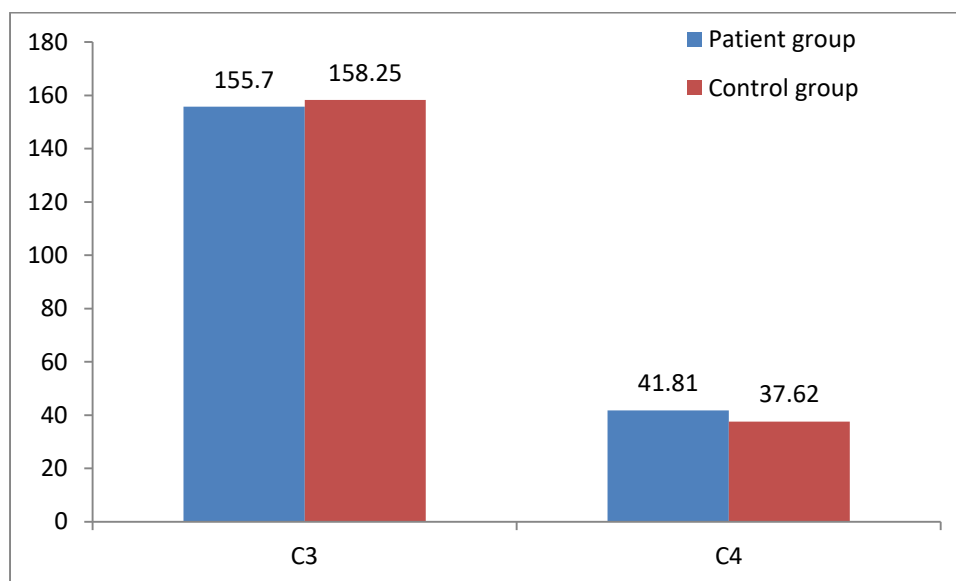
N	C ₃		C ₄	
	diameter	concentration	diameter	concentration
1	8.2	146.3	9	84
2	5.3	51.3	4	7.1
3	7.3	112.4	6	30.8
4	9.2	188.5	6.5	38.2
5	11.5	282.1	9.7	99.5
6	8.7	166.8	8	63.9
7	8	138.4	5.5	24
8	7.2	108.9	4.8	15.4
9	11	276.7	7	46.2
10	6.5	85.6	4.2	9
Average		155.7		41.81
Standard deviation		81.7753251		30.0758857



Scheme 3. Demonstrates the difference in C3 concentration for the control group and the patients.



Scheme 4. Demonstrates the difference in C4 concentration for the control group and the patients



Scheme 5. Demonstrates the difference in the mean C3 and C4 concentration of the patients control group.

CONCLUSION

- 1- High concentration of antibodies (IgA, IgM) in people with *B. Militance*
- 2- High concentration of immune proteins (C3, C4) in infected persons

REFERENCES

1. Pappas, G.; Bosilkovski, M.; Akritidis, N.; Tsianos, V.E. (2005). Brucellosis. *N. Engl. J. Med.* 352, 2325--2336.
2. Johansen, M.V.; Welburn, S.C.; Dorny, P.; Brattig, W.N. (2017). Control of neglected zoonotic diseases. *Acta Trop.* 165, 1--2.
3. Foster, J.T.; Walker, M.F.; Rannals, D.B.; Hussain, H.M.; Drees, P.K.; Tiller, V.R.; Hoffmaster, R.A.; Al-Rawahi, A.; Keim, P.; Saqib, M. (2017). African lineage *Brucella melitensis* isolates from Omani livestock. *Front. Microbiol.*, 8, 2702
4. Corbel M J. (1997). Brucellosis: an overview. *Emerging Infectious Diseases* 2: 213--21.
5. Lucero, N.E.; Escobar, I.G.; Ayala, M.S.; Jacob, N. (2005). Diagnosis of human brucellosis caused by *Brucella canis*. *J. Med. Microbiol.* 54, 457--461.
6. Gatselis. N K, Konstantinos P. M. Ioannis G. A., Stefos K., George N. D. (2011). Unusual cardiovascular complications of brucellosis presenting in two men: two case reports and review of the literature. *Journal of medical case reports.*
7. Hussein, Areej Attia; Muhammad, Wafa Hussein; Ali, Shahnaz Abdul Qadir (2013). Measuring the level of IgG, IgM, and IgA antibodies and C3 and C4 complement proteins in children infected with the parasite *Girardia lamblia*. *Nahrain University Journal*, Volume 16, Issue 1, pp. 5-1.
8. Benjamini, E.; Coica R.; Sunshine G. (2000). *Immunology. A Short course* "4th Ed., Wiley - Liss, Inc., U.S.A. Pp: 70-80.
9. Roitt, I.; Brostoff J.; Male D. (2001). "Immunology" 6th Ed., Mosby, Spain, Pp: 343-260.

10. Stewart, J. (2002). "Parasitic infections pathogenesis and immunity" In: Medical microbiology, by Greenwood, David; Richard C.B. Slack and John F. Peutherer, 17th Ed., Printed in China by RDC Group Limited, Pp: 154-160.
11. Mili, N.; Auckenthaler, R.; Nicod, L.P. (1993). Chronic brucella empyema. *Chest* 103, 620--621.
12. Kose, S.; Serin Senger, S.; Akkoçlu, G.; Kuzucu, L.; Ulu, Y.; Ersan, G.; Oguz, F. (2014). Clinical manifestations, complications, and treatment of brucellosis: Evaluation of 72 cases. *Turk. J. Med. Sci.* 44, 220--223.
13. Gunduz, T.; Tekturk, T.P.; Yapici, Z.; Kurtuncu, M.; Somer, A.; Torun, M.S.; Eraksoy, M. (2017). Characteristics of isolated spinal cord involvement in neurobrucellosis with no corresponding MRI activity: A case report and review of the literature. *J. Neurol. Sci.*, 372, 305--306.
14. Dias, S.P.; Sequeira, J.; Almeida, M. (2018). Spastic paraparesis and sensorineural hearing loss: Keep brucellosis in mind. *J. Neurol. Sci.* 385, 144--145.
15. Ducrottoy, M.J.; Muñoz, M.P.; Conde-Álvarez, R.; Blasco, M.J.; Moriyón, I. A. (2018). Systematic review of current immunological tests for the diagnosis of cattle brucellosis. *Prev. Vet. Med.* 151, 57--72.
16. Sharda, D.C.; Lubani, M. (1986). A study of brucellosis in childhood. *Clin. Pediatr.* 25, 492--495.
17. Pappas, G.; Bosilkovski, M.; Akritidis, N.; Tsianos, V.E. (2005). Brucellosis. *N. Engl. J. Med.* 352, 2325--2336.
18. Sedy, J. R.; Gavrieli, M.; Potter, K. G.; Hurchla, M. A.; Lindsley, R. C.; Hildner, K.; et al. (2005). B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. *Nat Immunol* 6 (1): 90-98.