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Natural resistance against brucellosis

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Abstract
Selective breeding of superior individuals were preferred earlier for the genetic up gradation of livestock. With advancements in molecular genetics and biotechnology, the economically important candidate genes *viz.*, genes responsible for resistance or susceptibility in pathogens, have recently been identified, and characterized in animals and can be appended for selection.
Considering the potential application of suitable genetic markers as discussed herein for resistance against bovine brucellosis, the present study was to investigate the influence, role, and correlated and individual outcome of the different factors, candidate genes, and genetic markers involved in *Brucella* infection resistance or/and susceptibility in cattle.

Keywords: Natural disease resistance, Genetic factors, Animal Brucellosis

Introduction
Brucellosis, an intracellular bacterial zoonosis, is distributed globally as a major issue of public health and economic concern and is a potential biowarfare agent. Brucellosis, primarily a disease of animals, is transmitted to humans by direct contact or indirect means (Skendros and Boura 2013, Agasthya A, *et al.*, 2007) [1, 2]. Despite the successful control of brucellosis in some of the countries of the Northern hemisphere, it is an issue of major concern worldwide and predominant in many developing countries (Brahmbhatt M. *et al.*, 2009, Paixao T. *et al.*, 2007, Ariza J. *et al.*, 2007, Adesiyun A. *et al.*, 2011, Feng J. *et al.*, 2015) [3-7].
Undoubtedly, *B. Melitensis* outbreaks cause considerable losses in terms of abortion, loss of milk production, reduced meat production due to the birth of a weak newborn, and expenses on maintaining the nonproductive animals. Human brucellosis causes physical and psychological suffering, the cost of drugs and hospitalization, and work loss due to illness. Still, we don't have an efficacious vaccine available against human brucellosis.
With existing socio-economic conditions in India, as test and slaughter cannot be practiced, and thus the control solemnly relies on sanitary control and herd management, test and segregation, treatment of infected and vaccination, and the gradual building up of herd immunity (Ranjan S. *et al.*, 2014) [8]. With the current technology and control strategies (e.g., antibacterials and vaccination), it is unrealistic to expect substantial decreases in brucellosis prevalence in animals and subsequently zoonoses in human in the near future, thus the losses attributable to the livestock industry are persistent. Instead, vaccinations induce havoc in livestock and indiscriminate use of antimicrobials makes microbes resistant. However, complete eradication is not possible, therefore, there is a demand to develop replacements to fight, manage and control infectious diseases. Still on, improvement of the overall genetic resistance to infectious diseases at the herd and population level by selective breeding programs could be the best and permanent substitute. Indeed, to select resistant cattle, the identification of genes associated with a natural resistance against brucellosis has been extensively investigated and, the older recovered animals were kept in the herd with the intention to provide some resistance/immunity against future infection (Adams L. and Templeton J, 1998, Paixao T, *et al.*, 2007, White P, *et al.*, 2013, Ranjan S, *et al.* 2014) [9, 4, 10, 8].
In view of using genetic natural resistance as a management tool counts on to discern effectively susceptible/resistant animals and its applicability to the population, here we briefly report the genes responsible for the natural resistance against brucellosis and the selection of the resistant animals.

Brucella Infection
*Brucella*e are small, non-motile, gram-negative, facultative intracellular coccobacillus that can survive in a broad range of host cells, particularly in mononuclear phagocytic cells or

macrophages, and belongs to the alpha 2 subdivision of class Proteobacteria. The intracellular behavior of these species confines manifestation to innate as well as the acquired immune system (Smith L, Fitch T, 1990, Ranjan S, *et al.*, 2014, Feng J, 2014, Figueiredo P, *et al.*, 2015) [11, 8, 7, 12].

There are six classical species of *Brucella viz*., *B. melitensis* (goats), *B. Abortus* (cattle), *B. Suis* (swine), *B. canis* (dogs), *B. Ovis* (sheep) and *B. Neotomae* (desert mice); new species *B. cetaceae* (cetacean) and *B. Pinnipediae* (seal) have recently been introduced. The first four species are of major zoonotic importance affecting human and have been listed as CDC/NIAID category B priority pathogens and notified as priority agents amenable for biological warfare and bio-terrorism use (Smith L, Fitch T, 1990, Xiang Z, *et al.*, 2006, and White P *et al.*, 2013) [11, 13, 9].

A strong tissue tropism (liver, spleen, bone marrow, lymph nodes, etc. reticuloendothelial system) betrayed by *Brucella* spp. and they reproduce within macrophage-, placental trophoblast-and dendritic- cell's vacuoles to result into an infection. Although they are transmitted by ingestion get endocytosed intact by macrophages and neutrophils through the gastrointestinal epithelium. They survive in the reticuloendothelial system representing the tissue tropism and relapsing nature of the infection. The infection involves nonspecific inflammation which gives rise to detectable antibodies in 1 to 2 weeks post-infection. The infection, as revealed by pathology, can be of three phases incubational-, acute- and chronic- phase (Smith L, Fitch T, 1990, Lim M, *et al.*, 2014, Figueiredo P, *et al.*, 2015) [11, 14, 12].

The primary cellular target of *Brucella* spp. is phagocytic macrophages and nonphagocytic epithelial cells (Ferrero M, *et al.*, 2013, Skendros P and Boura P 2013) [15, 1]. The virulence depends upon the capability to survive and replicate intracellularly. The progression of infection may be influenced by age, sex and/or natural resistance. Heifers from infected cows were found seronegative for long time which may act as reservoir of infection (Smith L, Fitch T, 1990, Bercovich Z, *et al.*, 1990 and Xiang Z, *et al.*, 2006) [11, 16, 13].

Buffaloes and bison are believed to be naturally resistant to some extent to *Brucella-induced* abortions as they have high heat and stress forbearance, which might be due to their powerful innate immune system (Herman J, 2013, Moreno E, 2014, Patel S, *et al.*, 2015) [17-19].

Mechanisms of natural resistance

The inherent ability of an animal (or living being) to resist a particular disease when exposed to the pathogen (without prior immunization or exposure) is referred to as natural disease resistance (Westhusin M, *et al.*, 2015) [20]. Innate and adaptive immunity are two classical functional divisions. Innate immunity is the first line of defense against invading pathogens, evaluated as a consequence of natural selection in livestock. Whereas the adaptive response is acquired by an individual as a result of exposure to respective pathogens which is further subdivided into cellular and humoral immunity (Skendros P, Boura P 2013, Patel S, *et al.*, 2015) [1, 19].

Host responses to brucellosis are variable and complex, dependent on host species, species or strain of *Brucella* and intensity of exposure. Different animal species demonstrate biological differences in immunity such as antibody production, lymphocyte performance, and macrophage or susceptibility genes. Differences in disease susceptibility and immune response may be elaborated by external factors like environment and management practices, or by internal factors like nutrition and genetics. Consequently, it is often necessary to correlate and balance functional traits of breeding strategies for high production with health and disease resistance traits (Skendros P, Boura P, 2013, Ranjan S, *et al.*, 2014) [1, 10].

Humoral and cell-mediated immunity both play a significant role in *B. abortus* susceptible and resistant cattle and both are important in resistance to bovine brucellosis (Price R, *et al.*, 1990) [21].

The acquired cell-mediated resistance and macrophage activation by gamma interferon (IFN- γ) producing T lymphocytes regulate the resistance against facultative intracellular bacteria like *Brucella* spp. Macrophage-derived cytokines, particularly interlukin-12 (IL-12), significantly induce $CD4^+$ T cells to produce IFN-γ, however, IL-12 also involved in NK cell stimulation to produce IFN- γ. The IL-2 induced T and NK cells activation contributes to the resistance against *Brucella* (Skendros P, Boura P, 2013, Zhan Y, Cheers C, 1995 and Ellergezen P, *et al.*, 2023) [1, 22, 23].

Unwinding the role of genetics in disease susceptibility and immune response in various species is of persistent interest in livestock and wildlife management. How many genes are involved essentially in intracellular resistance and how they interact, the picture still is unpredicted (Xiang Z, *et al.*, 2006) [13].

Zhan Y and Cheers C. (1995) [22] reported the IL-12 production during *B. Abortus* infection promotes the splenic T cell IFN-γ production and increases macrophage activity that ultimately resulted in bacteria clearance.

Host genes involved in resistance

The valuable connexion between natural resistance to virulent *B. Abortus* and different genes enables the selection and breeding of naturally resistant domestic and free-ranging ungulates which instead could play a significant temporal and resultant role to develop a new strategy to effectively control these worldwide important zoonotic diseases (Feng J, *et al.*, 2015) [7]. Identification of the concerned gene against brucellosis resistance has been investigated extensively to choose resistant animal species (Paixao T, *et al.*, 2012) [4].

Bovine innate immune system involves beta-defensins (DEFB1, BNBD4, BNBD5, TAP- Tracheal Antimicrobial Peptide), cathelicidin, toll-like receptors, chromatogranins (A, B, C) and NRAMP (NRAMP1, NRAMP2), etc. (Skendros P, Boura P, 2013, Patel S, *et al.*, 2015) [1, 19].

Among the various genetic markers and gene candidates recently identified and characterized (Solute Carrier family11 member A1 (SLC11A1 formerly NRAMP1) gene, Major Histo Compatibility (MHC) genes, Toll-Like Receptor *(TLR)* genes etc.) involved in resistance/susceptibility to infections with various pathogens including *Brucella*, *SLC11A1* gene plays vital role in human and various livestock species (Thomas N, Joseph S, 2012 and Patel S, *et al.*, 2015) [24, 19].

Natural-Resistance Associated Macrophage Protein 1 (Nramp1 gene)

The Slc11a1 (solute carrier family 11 member A1) (formerly *Nramp1* gene - coding for natural resistance-associated macrophage protein 1), a candidate gene associated with resistance against intracellular pathogens, was studied extensively in many species and first recognized in mice, previously known as *Lsh/Ity/Bcg* (Wyllie S, *et al.*, 2002,

Barshes N, *et al.*, 2006 and Paixao T *et al.*, 2007) [25, 26, 4]. The 3' untranslated region (UTR) sequences of the *NRAMP* gene supports the phylogenetic relationship between mitochondrial DNA and nuclear DNA analyses (Thomas N and Joseph S. 2012) [24].

The Slc11a1 is a major candidate gene with determining role in the outcome of infections caused by intracellular pathogens in numerous animal species, highly conserved in mammals and some conservation found also in mice and human gene structure (Coussens P, *et al.*, 2004, Hasenauer F, *et al.*, 2022) [27, 28], which confers resistance against bovine brucellosis (Westhusin M, *et al.* 2015) [20], encodes an integral membrane protein involved in the regulation of macrophage activity (Thomas N and Joseph S 2012) [24].

The polymorphism in NRAMP1 gene association in cattle has been limited to a single microsatellite in the 3' untranslated region (3'UTR) (GT) n microsatellite of the *NRAMP1* gene (variation in the number of GT repeats). These polymorphisms correspond to a variation in the number of GT repeats (13 to 16 GT repeats have been identified), involved in a polymorphic (GT) n microsatellite located at the 3'UTR. These polymorphisms are involved in increased HO (hydrogen peroxide) and NO (nitrous oxide) production which markedly is associated with improved macrophage activity in buffalo (Thomas and Joseph 2012) [24]. It also mingled in phagosome acidification and phagosome lysosome fusion in macrophages and is thus responsible for some intracellular infections and autoimmune diseases (Seabury C. *et al.*, 2005) [29].

However, (GT)13 allele was found to be associated with natural resistance to bovine brucellosis, whereas no association of resistance to *Brucella* corresponding to NRAMP1 3'UTR polymorphism was recorded in humans (Adams L and Templeton J, 1998, Paixao T, *et al.*, 2007) [8, 4].

The polymorphisms in the bovine *Slc11a1* gene when demonstrated, and screened, have been found to have high allelic diversity, and in turn, correlated with the resistance or susceptibility to specific diseases in cattle including brucellosis (Boonyaprakob U and Homsavart S. 2014) [30], which could be used in selection and breeding animal for disease resistance. Cattle have a polymorphism of the Nramp1 gene which may be responsible for the enhanced ability to control intracellular *B. Abortus* in monocytes derived from naturally resistant cattle (Feng J. *et al.*, 2015) [7]. The polymorphisms in the bovine *Slc11a1* gene was demonstrated in different Indian cattle breeds (Ranjan, R. *et al.*, 2011, Ranjan R, *et al.*, 2015) [31-32].

Slc11a1 have been demonstrated to influence the intraphagosomal replication of microbes which in turn confer intracellular pathogens and also reported to prevent the bacterial growth in macrophages by modulation of iron (Fe) metabolism in macrophages plays a vital role in host innate immunity and also affects adaptive immunity with its pleiotropic effects and cytokine mRNAs stabilization (Wyllie S, *et al.*, 2002, Paixao T, *et al.*, 2007, Boonyaprakob U and Homsavart S. 2014,) [25, 4, 30].

In contrast to the data presented here, a study by Paixao T. *et al.*, (2007) did not reported any association between a 3'UTR polymorphism of *SLC11A1* gene and resistance to brucellosis in cattle. Again, Kumar N, *et al.*, (2011) [33] denoted *TaqI* and *AluI* polymorphisms in and around TM4 (transmembrane domain 4) of *SLC11A1* gene in cattle but an association with the brucellosis resistance/susceptibility could not be established (Thomas N. and Joseph S. 2012) [24]. Interleukin-8 receptor linked to bovine NRAMP1 gene (Feng J, *et al.*, 2015) [7].

Toll-like receptor 2 (TLR2)

Toll-like receptor 2 (TLR2) functional in innate as well as adaptive immune systems, is one of the significant pattern recognition receptors which has been found as a susceptibility locus for several infections by activating, vitally, innate immune responses (Yapan S, *et al.*, 2014, Radhakrishnan G, *et al.*, 2009) [35, 34]

Pathogenic microbes directly interfere with TLR action with the inhibitory homolog secretions of Toll/interlukin-1receptor (TIR) domain, as one of the strategies to subvert the host immune system (Radhakrishnan G, *et al.*, 2009) [34]. The TIR-like domains are widespread in bacteria as close homologs were reported in unrelated bacterial species, suggesting their lateral transfer (Newman R, *et al.*, 2006) [36].

TLR2, TLR4, and TLR9 in cognition with macrophages and dendritic cells, were demonstrated to be involved in the recognition of *Brucella.* TLR2 recognized *Brucella* antigens induce proinflammatory cytokine synthesis leading to bacterial clearance and, unlike that, MHC-II downregulation boon bacterial persistence (Newman R, *et al.*, 2006, Ferrero M, *et al.*, 2013) [36, 15]. Therefore, TLR2 recognition was thus stated to be complexed with chemokine/cytokine response of alveolar macrophages to *Brucella* infection, MHC-II expression down regulation, *Brucella* induced antigen presentation and antigens in macrophages (Ferrero M. *et al.*, 2013) [15]. The balance between the two roles discussed above controls the output of TLR2 recognition system on *Brucella* survival.

Interlukin-12

IL-12 induces $CD4^+$ T cells and stimulates NK cells to produce IFN-γ which vitally control the infection. IL-12 produced during infection promotes IFN-γ synthesis and *in vivo* clearance of bacteria. For the control of intracellular bacterial infection, IFN-γ and positive regulators of cellular immunity are dominant (Zhan Y. and Cheers C. 1995) [23].

MHC (Major Histocompatability Complex)

However, Sathiyaseelan *et al.*, 2000 [37] proposed to focus on differences in the expression of cytokine genes and their receptors especially involved in macrophages activation.

PRNP (Prion Protein Gene)

The interaction of heat shock protein and host-encoded cellular prion protein (PrP^C) has recently been manifested, notifying it as a significant cell surface receptor/ portal protein receptor for *B. abortus* in mice. Mice PrP^C facilitated evaluation of nucleotide and amino acid variation in exon 3 of PRNP in bison populations (Herman J, 2013, Seabury C, *et al.*, 2005) [17, 29].

Seabury C, *et al.*, (2005) [29] reported the significant association between nucleotide variation in PRNP exon 3 sequence and *Brucella* spp. antibodies in bison, the pinpointing relationship between PrP^C and *Brucella* infection, and its role in natural disease resistance in bison.

References

1. Skendros P, Boura P. Immunity to brucellosis. Rev Sci tech off int Epiz. 2013;32(1):137-147.

2. Agasthya AS, Isloor S, Prabhudas K. Brucellosis in high risk group individuals. Ind. J Med. Microbiol. 2007;25:28-33.
3. Brahmabhatt MN, Varasada RN, Bhong CD, Nayak JB. Seroprevalence of Brucella spp. in buffaloes in the central Gujarat region of India. Buff. Bulletin. 2009;28:73-75.
4. Paixao TA, Poester FP, Carvalho Neta AV, Borges AM, Lage AP, Santos RL. NRAMP1 3 Untranslated Region Polymorphisms Are Not Associated with Natural Resistance to *Brucella* abortus in Cattle. Infec. Immun. 2007;75(5):2493-2499. DOI: 10.1128/IAI.01855-06
5. Ariza J, Bosilkovski M, Cascio A, Colmenero JD, Corbel MJ, Falagas ME, *et al*. Perspectives for the Treatment of Brucellosis in the 21st Century: The Ioannina Recommendations. 2007;4(12):e317.
6. Adesiyuyn AA, Baird K, Stewart-Johnson A. Antimicrobial resistance, phenotypic characteristics and phage types of *B. abortus* strains isolated from cattle and water buffalo (Bubalus bubalis) in Trinidad. Vet archive. 2011;81:391-404.
7. Feng J, Li Y, Hashad M, Schurr E, Gros P, Adams LG, *et al*. Bovine natural resistance associated macrophage protein 1 (Nramp1) gene. Genome Res. 1996;6:956-964.
8. Ranjan S, Sharma A, Chopra A, and Mitra A. Transcript abudance of bovine NRAMP1 and iNOS genes among *Brucella* sero-reactive cattle and buffalo. Afr. J. Microbiol. Res. 2014;8:1850-1854. DOI: 10.5897/AJMR2014.6624
9. Adams LG, Templeton JW. Genetic resistance to bacterial diseases of animals. Rev. Sci. tech. Off. Int. Epiz. 1998;17:200-219.
10. White PJ, Treanor JJ, Geremia C, Wallen RL, Blanton DW, Hallac DE. Bovine brucellosis in wildlife: using adaptive management to improve understanding, technology and suppression. Sci. Tech. Off. Int. Epiz. 2013;32(1):263-270.
11. Smith LD, Ficht TA. Pathogenesis of Brucella. Crit Rev Microbiol. 1990;17(3):209-230. DOI: 10.3109/10408419009105726
12. Figueiredo P de, Ficht TA, Rice-Ficht A, Rossetti CA, Adams LG. Pathogenesis and Immunobiology of Brucellosis Review of Brucellae Host Interactions. The American J Path; c2015. p. 185. DOI: http://dx.doi.org/10.1016/j.ajpath.2015.03.003
13. Xiang Z, Zheng W, He Y. BBP: Brucella genome annotation with literature mining and Curation. BMC Bioinformatics. 2006;7:1-14.
14. Lim ML, and Rickman LS, Brucellosis. Infect. Dis. Clin. Pract. 2004;12:7-14.
15. Ferrero MC, Hielpos MS, Carvalho NB, Barrionuevo P, Corsetti PP, Giambartolomei GH, *et al*. Key Role of Toll-Like Receptor 2 in the Inflammatory Response and Major Histocompatibility Complex Class II Downregulation in *Brucella abortus*-Infected Alveolar Macrophages. Inf. Immun; c2014. p. 82. DOI: 10.1128/IAI.01237-13.
16. Bercovich Z, Haagsma J, Ter Laak EA. Use of delayed-type hypersensitivity test to diagnose brucellosis in calves born to infected dams. Vet. Quarterly. 1990;12:231-237.
17. Herman, JA. Genetic natural resistance to brucellosis in Yellowstone National Park bison (Bison bison): A preliminary assessment. Colorado State University, Thesis. 2013;106:1539551.
18. Moreno E. Retrospective and prospective perspectives on zoonotic brucellosis. Front. Microbiol. 2014;5:213. DOI: 10.3389/fmicb.2014.00213
19. Patel SM, Koringa PG, Nathani NM, Patel NV, Shah TM, Joshi CG. Exploring genetic polymorphism in innate immune genes in Indian cattle (Bos indicus) and buffalo (Bubalus bubalis) using next generation sequencing technology. Meta Gene. 2015;3:50-58. DOI: http://dx.doi.org/10.1016/j.mgene.2015.01.002
20. Westhusin ME, Shin T, Templeton JW, Burghardt RC, Adams LG. Rescuing valuable genomes by animal cloning: A case for natural disease resistance in cattle. J. Anim. Sci. 2007;85:138-142. DOI: 10.2527/jas.2006-258
21. Price RE, Templeton JW, Smith IIIR, Adams LG. Ability of mononuclear phagocytes from cattle naturally resistant or susceptible to brucellosis to control *in vitro* intracellular survival of *Brucella* abortus. Infec. Immun. 1990;58:879-886.
22. Zhan Y, Cheers C. Endogenous Interleukin-12 is involved in Resistance to *Brucella* abortus Infection. Infect Immun. 1995;63:1387-1390.
23. Ellergezen PH, Kizmaz MA, Simsek A, Demir N, Cagan E, Bal SH, *et al*. Investigation of IL-35 and IL-39, New Members of the IL-12 Family, in different clinical presentations of Brucellosis. Immunol. Invest; c2023. p. 1-12 DOI: doi.org/10.1080/08820139.2023.2165941
24. Thomas N, Joseph S. Role of SLC11A1 gene in disease resistance. Biotech in Ani. Husb. 2012;28(1):99-106.
25. Wyllie S, Seu P, Goss JA. The natural resistance-associated macrophage protein 1 Slc11a1 (formerly Nramp1) and iron metabolism in macrophages. Microbes. Infect. 2002;4:351-359.
26. Barshes NR, Lee TR, Goss JA, Goodpastor SE, Huls MH, Rooney CM, *et al*. Slc11a1 (formerly Nramp1) polymorphisms and susceptibility to post-transplant lymphoproliferative disease following pediatric liver transplantation. Transplant Inf. Dis; c2006. p. 10. 1111/j.1399-3062.2006.00139.x
27. Coussens PM, Coussens MJ, Tooker BC, Nobis W. Structure of the bovine natural resistance associated macrophage protein (NRAMP 1) gene and identification of a novel polymorphism. DNA Seq. 2004;15:15-25.
28. Hasenauer FC, Caffaro ME, Poli MA, Rossetti CA. Evaluation of polymorphisms at the 3´ UTR SLC11A1 gene microsatellites and their associations with the outcome of Brucella abortus infection in *Bos taurus* cattle. Ediciones INTA; c2022.
29. Seabury CM, Halbert ND, Gogan PJP, Templeton JW, Derr JN. Bison PRNP genotyping and potential association with Brucella spp. Seroprevalence. Ani. Genet. 2005;36:104-110. DOI: 10.1111/j.1365-2052.2005.01240.x
30. Boonyaprakob U, Homsavart S. Molecular characterization of a cDNA encoding Slc11A1 (Nramp1) of native Thai cattle. Nat. Sci. 2014;48:52-63.
31. Ranjan R, Bhong CD, Chavan KV, Parmar SNS, and Joshi CG. DNA polymorphism of 3'UTR of Nramp1 gene in Malvi breed of cattle. J Adv. Vet. Res. 2011;1:105-108.
32. Ranjan R, Bhong CD, Parmar SNS, Joshi CG. Molecular characterization of 3'UTR of Nramp1 gene in Gaolao breed of cattle. Ind. J Ani. Res. 2015;49:31-35.
33. Kumar N, Ganguly I, Singh R, Deb SM, Kumar S,

Sharma A, *et al*. DNA polymorphism in SLC11A1 gene and its association with brucellosis resistance in Indian zebu (Bos indicus) and crossbred (Bos indicus × Bos taurus) Cattle. Asian-Australasian J Ani. Sci. 2011;24:898-904.
34. Radhakrishnan GK, Yu Q, Harms JS, Splitter GA. *Brucella* TIR domain-containing protein mimics properties of the toll-like receptor adaptor protein TIRAP. J Biolog Chem. 2009;284(15):9892-9898. DOI: 10.1074/jbc.M805458200
35. Yapan, S., Liping S, Aizhen G, Liguo Y. Effects of toll-like receptor 2 gene mutation on resistance to bovine brucellosis. Livestock Sci. 2014;170:30-34.
36. Newman RM, Salunkhe P, Godzik A, Reed JC. Identification and characterization of a novel bacterial virulence factor that shares homology with mammalian Toll/interleukin-1 receptor family proteins. Infec. Immun. 2006;74(1):594-601. DOI:10.1128/IAI.74.1.594–601.
37. Sathiyaseelan J, Jiang X, Baldwin CL. Growth of *Brucella* abortus in macrophages from resistant and susceptible mouse strains. Clin. Exp. Immunol. 2000;121:289-294.