

Neurobrucellosis with Acquired Hemophagocytic Lymphohistiocytosis in a Two-Year-Old Bolivian Male

JAMES L. RAZER, CATERINA M. MIRAGLIA

ABSTRACT

Hemophagocytic lymphohistiocytosis (HLH) is a rare hyper-inflammatory syndrome that may be inherited or acquired. HLH may be a complication of infectious disease, including brucellosis. Central nervous system involvement in brucellosis is uncommon, occurring in only 5-7% of cases. In this report we describe a case of neurobrucellosis with acquired HLH in a two-year-old Bolivian male. The patient presented to the emergency department with recurrent fevers, malaise, anorexia, and abdominal distention. HLH was suspected due to hepatosplenomegaly, fever, and evidence of hemophagocytosis in a previous bone marrow biopsy. Pancytopenia, increased ferritin, and increased transaminases were observed in laboratory workups, which along with hepatosplenomegaly are consistent with HLH. Positive blood cultures and PCR identification of *Brucella melitensis* indicated the cause of HLH. Magnetic resonance imaging (MRI) results and analysis of cerebrospinal fluid indicated neural involvement. Treatment of neurobrucellosis with rifampin, gentamicin, doxycycline, and ceftriaxone resolved the infection, which in turn resolved the HLH. This case demonstrates the importance of considering brucellosis as a differential diagnosis of HLH.

ABBREVIATIONS: HLH - Hemophagocytic lymphohistiocytosis, WBC - White blood cell, RBC - Red blood cell, ALT - Alanine aminotransferase, AST - Aspartate aminotransferase, ALP - Alkaline phosphatase, LDH - Lactate dehydrogenase, PCR - Polymerase Chain Reaction, CBC - Complete blood count, TNF- α - Tumor necrosis factor- α

INDEX TERMS: Hemophagocytic lymphohistiocytosis, neurobrucellosis, *Brucella melitensis*

Clin Lab Sci 2016;29(3):131-137

James L. Razer, B.S., MLS(ASCP)^{CM}, Boston Children's Hospital, Boston, MA

Caterina M. Miraglia, D.C., MLS(ASCP)^{CM}, Department of Medical Laboratory Science, University of Massachusetts, Dartmouth, MA

Address for Correspondence: Caterina M. Miraglia, D.C., MLS(ASCP)^{CM}, Department of Medical Laboratory Science, University of Massachusetts Dartmouth, 285 Old Westport Road, Dartmouth, MA 02747, 508-999-8584, caterina.miraglia@umassd.edu

CASE REPORT

A developmentally normal two-year-old male with a previous history of infection including *Rotavirus* and *Giardia* spp., presented with recurrent fevers. Over the course of three weeks after the onset of fevers, the patient became progressively lethargic with decreased appetite and abdominal distention. The patient had resided in Santa Cruz, Bolivia and his only animal contact was with the family dog. Bolivian physicians performed an initial bone marrow biopsy that did not show leukemia, but did reveal hemophagocytosis. They referred the patient to a hospital in the United States. There, the patient's parents noted a history of low WBC counts, anemia, and thrombocytopenia. To confirm the Bolivian data, a CBC with differential, prothrombin time, partial thromboplastin time, international normalized ratio, estimated fibrinogen and complete metabolic panel were ordered.

The admission CBC and differential revealed pancytopenia and a left shift as represented in Table 1. The chemistry laboratory results shown in Table 2 demonstrated increased transaminases, LDH, ALP, C-reactive protein, and triglycerides, as well as a

markedly increased ferritin. These results, along with the hepatosplenomegaly observed during physical examination were strongly suggestive of HLH. Characteristic signs of HLH include fever, hepatosplenomegaly, and cytopenia in at least two cell lines.^{1,2} Other laboratory findings seen in HLH include elevated triglycerides, ferritin, transaminases, bilirubin, and decreased fibrinogen.² Activated macrophages secrete ferritin and plasminogen activator, accounting for the patient's increased ferritin and abnormal coagulation results [Table 2, 3].¹ Increased inflammatory cytokines are responsible for observed cytopenias, elevated triglycerides, and decreased fibrinogen.² A bone marrow biopsy was ordered for confirmation, which displayed markedly increased macrophages, decreased myeloid to erythroid ratio, and hemophagocytosis of nucleated erythroid precursors. (Figure 1) It was negative for microorganisms. The patient was started on immunosuppressants and chemotherapeutic agents to control the symptoms of HLH until the cause was identified. Treatment included anti-thymocyte globulin and etoposide. The focus was then placed upon determination of the cause of HLH.

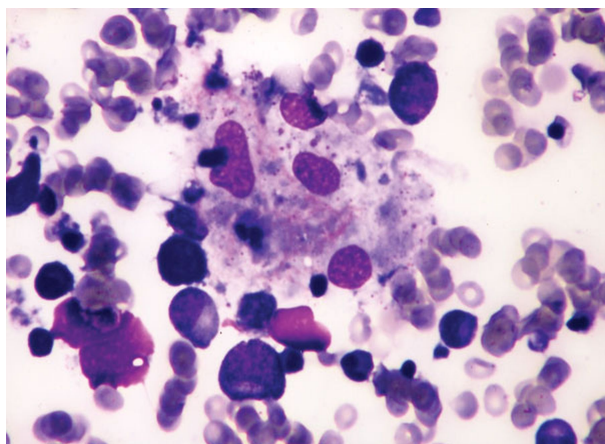


Figure 1. Wright stain x400. Hemophagocytosis of platelets, nucleated red blood cells, and a neutrophil by a histiocyte in a bone marrow aspirate. The image is courtesy of Dr. Je- Jung Lee and is available at <http://wwwnc.cdc.gov/eid/article/8/2/01-0299-f1>.

HLH is a prolonged hyper-inflammatory state that can be inherited or it can be acquired through different mechanisms, including infectious agents.^{1,2} Genetic testing with an HLH sequencing panel and targeted deletion and duplication assay for HLH

were both negative [Table 4]. This demonstrated that the patient's presentation of HLH was not of familial origin. Toxins and rheumatic diseases as potential causes were ruled out by chemistry results and lack of evidence upon physical examination, which then directed laboratory testing to infectious disease agents. Empiric therapy of vancomycin and piperacillin-tazobactam was administered due to the suspicion of infection with endemic dimorphic fungi. A cerebrospinal fluid specimen was analyzed for total protein, glucose levels, and differential. The total protein was increased and the glucose was decreased. The differential results were as follows: 90 lymphocytes, 1 neutrophil/band, 2 macrophages, and 7 monocytes out of 100 cells counted. Magnetic resonance imaging was ordered due to the patient exhibiting an altered mental state and weakness. It showed an abnormality primarily in the subcortical region as well as the periventricular regions. Blood, stool, bone marrow, and urine specimens were obtained for testing of infectious disease agents. All sample manipulations were performed in a biosafety cabinet utilizing Biosafety Level 3 precautions. After 4 days of incubation, small "grain of sand" like Gram-negative coccobacilli were observed on a Gram stain from an aerobic blood culture. (Figure 2) All other infectious disease tests were negative [Table 5]. The isolated organism was identified as presumptive *Brucella* spp. at the state laboratory by PCR, and then later confirmed as *Brucella melitensis* by the Centers for Disease Control and Prevention.



Figure 2. *Brucella melitensis*. Note the small "grain of sand" appearance of the Gram-negative coccobacilli. The image is courtesy of the CDC Public Health Image Library (ID#1937) and is available at <http://phil.cdc.gov/phil/details.asp>.

CLINICAL PRACTICE

Brucellosis requires a treatment regimen with antimicrobials that can be delivered to infiltrated tissue areas, possess the ability to penetrate the cell membrane of infected macrophages, and maintain activity in the acidic pH compartments where the organisms replicate.³ To accomplish this, a combination of antimicrobials is required. The World Health Organization recommendation for treatment of brucellosis is a regimen of doxycycline and either rifampin or streptomycin.³ These combinations are the most widely used treatment options, although combinations of doxycycline with trimethoprim-sulfamethoxazole or amino glycosides have also been found to be effective.³ Neurologic involvement requires a treatment plan with two to three antimicrobials.⁴ The use of streptomycin in neurobrucellosis is contraindicated due to the inability of the drug to reach therapeutic ranges within the central nervous system.⁵ Once the identification of *Brucella melitensis* was obtained, immunosuppressant treatment was halted and specific antimicrobial treatment was administered. The physician noted that treatment with three antimicrobials for neurobrucellosis was preferable due to lesser chance of relapse. Because of the severity of the patient's presentation, quadruple therapy with doxycycline, gentamicin, rifampin, and ceftriaxone was utilized. The combination of these four antimicrobials quickly began to positively change the patient's clinical status.

DISCUSSION

Brucellosis is the most common zoonotic disease worldwide. Infection occurs through direct contact with an infected animal, consumption of unpasteurized dairy products, or inhalation of infectious aerosolized particles, which is of particular concern for laboratory scientists.³ Upwards of 500,000 new cases are reported each year.^{6,7} While cases of human brucellosis in the United States are rare at an incidence of 0.4 cases per million annually, the disease is much more common in areas surrounding the Mediterranean sea, as well as the Middle East, Asia, Mexico, and South America, where the disease is endemic.⁷ Cases in the United States may be acquired by travel to an area where the organism is endemic. Cases in the U.S. have also been described in California and Texas due to consumption of raw dairy products brought in from

Mexico. These two states account for many of the cases in the U.S. where the disease is found mostly within the Hispanic population.^{5,8,9}

Table 1. Hematology Complete Blood Count and Differential Results

Assay	Patient	Reference Range
WBC	3.35x10 ⁹ cells/L L	5.97-10.49 x 10 ⁹ cells/L
HGB	94 g/L L	110-128 g/L
HCT	0.272 L/L L	0.315-0.368 L/L
PLT	26x10 ⁹ cells/L L	208-413 x 10 ⁹ cells/L
RBC	3.4x10 ¹² cells/L L	3.92-4.72 x 10 ¹² cells/L
MCV	80.1 fL	76.8-83.3 fL
MCH	27.6 pg	26.8-29.4 pg
MCHC	34.4 g/dL	34.2-36.7 g/dL
RDW	14.3%	12.0-14.5%
Reticulocyte count %	<0.5%	L 0.5 -1.5%
Absolute Neutrophil Count	1.58x10 ⁹ cells/L L	2.49-5.96 x 10 ⁹ cells/L
Left Shift	Present	Absent
Neutrophil/Band	47%	22-46%
Lymphocyte	45%	37-73%
Monocyte	2%	2-11%
Eosinophil	1%	1-4%
Basophil	1%	0-2%
Atypical lymphocyte	4%	0-4%

Low (L), White blood cell (WBC), Hemoglobin (HGB), Hematocrit (HCT), Platelet (PLT), Red blood cell (RBC), Mean corpuscular volume (MCV), Mean cell hemoglobin (MCH), Mean cell hemoglobin concentration (MCHC), Red cell distribution width (RDW).

The etiology of brucellosis is infection with members of the genus *Brucella*. These organisms are aerobic facultative intracellular Gram-negative coccobacilli, with "grains of sand" like appearance when Gram stained. These organisms belong to the class α -Proteobacteria within the phylum Proteobacteria, along-side intracellular pathogens such as *Rickettsia* spp. and *Bartonella* spp.¹⁰ Organisms of the genus *Brucella* are speciated based upon their natural reservoirs. *Brucella melitensis* is found in sheep and goats, *Brucella abortus* in cattle, *Brucella suis* in pigs, and the reservoirs for *Brucella canis* are dogs. All are human pathogens.^{3,6,10,11} *Brucella melitensis* is the most common human pathogen of the genus and was at one time believed to cause the most severe presentation of the disease. According to a recent study comparing severity of disease states between *Brucella abortus* and *Brucella melitensis* infection,

CLINICAL PRACTICE

there was no difference in clinical presentation.^{3,6}

Table 2. Chemistry Laboratory Test Results

Assay	Patient	Reference Range
Sodium	136 mmol/L	136 - 145 mmol/L
Potassium	3.73 mmol/L	3.5 - 5.1 mmol/L
Chloride	101 mmol/L	98 - 107 mmol/L
Glucose	4.5 mmol/L	4.2 - 5.8 mmol/L
Triglycerides	428 mg/dL H	Desirable: <250 mg/dL Mild: 250-500 mg/dL Severe: >500 mg/dL
BUN	3.2 mmol/L	1.8 - 6.4 mmol/L
Creatinine	17.7 mmol/L L	26.5 - 62 mmol/L
Total Protein	59 g/L	54 - 75 g/L
Albumin	29 g/L L	30 - 46 g/L
Total bilirubin	0.9 mg/dL	0.3 - 1.2 mg/dL
Direct bilirubin	0.4 mg/dL	0.0 - 0.4 mg/dL
AST	433 U/L H	2 - 40 U/L
ALT	122 U/L H	3 - 30 U/L
LDH	1,481 U/L H	110 - 295 U/L
ALP	512 U/L H	100 - 320 U/L
C-Reactive Protein	115.7 mg/L H	< 5 mg/L
Ferritin	14,325 ug/L H	10 - 75 ug/L

Blood urea nitrogen (BUN), Low (L), High (H), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH), Alkaline phosphatase (ALP).

Table 3. Coagulation Test Results.

Assay	Patient	Reference Range
PT	15.8 sec H	11.7-14.4 sec
INR	1.47 H	0.98 - 1.13
PTT	43.0 sec H	25.0-37.0 sec
Fibrinogen	<0.9 g/L L	2-4 g/L

High (H), Low (L), Prothrombin time (PT), International normalized ratio (INR), Partial thromboplastin time (PTT).

The classic method for isolation of *Brucella* spp. is with the use of Castaneda bottles, but the gold standard for identification of brucellosis is a bone marrow culture, where greater concentrations of the organism can be obtained.³ The intracellular nature of this organism makes isolation on routine media extremely difficult. Serological tests are available for presumptive identification, but speciation requires specialized biochemical and phage testing or PCR.³ Brucellosis is the most commonly reported laboratory acquired bacterial infection.¹² Due to the low infective dose and the ability of the bacteria to be easily aerosolized, all laboratory work with specimens from a patient with suspected brucellosis should be performed in a biological safety cabinet

utilizing Biosafety Level 3 precautions.^{12,5}

Table 4. HLH Panel Genetic Tests

Gene	Location	Function
AP3B1	5q14.1	Intracellular vesicle trafficking
BLOC1S6	15q21.1	Intracellular vesicle trafficking
ITK	5q31-q32	IL2 induced T-cell kinase
LYST	1q42.1-q42.2	Lysosomal trafficking regulation
MAGT1	Xq21.1	Magnesium ion transportation
PRF1	10q21-22	Transmembrane pore formation for granzyme transportation
RAB27A	15q21	Protein transportation/GTPase mediated signal transduction
SH2D1A	Xp25	Protein binding/Lymphocyte Signal Transduction modification
SLC7A7	14q11.2	Amino acid transportation
STX11	6q24	Intracellular vesicle fusion
STXBP2	19p13	Intracellular vesicle trafficking
TNFRSF7(CD27)	12p13	TNF receptor
UNC13D	17q25	Vesicle maturation and exocytosis/Cytolytic granule excretion regulation
XIAP	Xp25	Apoptosis inhibition

*All Genetic testing, including targeted deletion and duplication assays, were negative. Targeted deletion and duplication assays were performed on the same genes as the HLH sequencing panel. All genetic tests performed were laboratory developed and approved under the Clinical Laboratory Improvement Amendments of 1988.

Brucella spp. most commonly enters the body via the digestive tract. It may also enter via the respiratory tract or through abrasions in the skin. After initial exposure, there is typically a 2-4 week incubation period, (it may range from 1 week – 2-3 months), before the onset of signs and symptoms.^{6,5} Signs and symptoms of brucellosis tend to be non-specific in nature, which may make diagnosis difficult. Common signs and symptoms include fever, weight loss, anorexia, malaise, arthralgia, and hepatosplenomegaly. Many different systems may be affected, such as the respiratory tract, osteoarticular system, cardiovascular system, gastrointestinal tract, genitourinary system, hepatobiliary system, and central nervous system.⁵ Central nervous system involvement is rare, occurring in only 5-7% of brucellosis cases.^{3,13}

Table 5. Infectious Disease Test Results

Blood	Stool	Urine	Bone Marrow
Aerobic blood cultures	Ova and Parasite	Histoplasma antigen	Leishmaniasis pathology
PCR: HSV, VZV, EBV, CMV, adenovirus, HHV-6	Cyclospora, Cryptosporidium, Isospora stain	Quantitative Coccidioides antigen EIA (send out)	Histopathology
Antibody for HSV, VZV, EBV, CMV	Giardia, Cryptosporidium stain		
Antigen and antibody for HIV-1 and HIV-2			
Strongyloides antibody			
Leishmaniasis serology			
Parasite smear			
Dengue serology			
Coccidioides antibody			
Cryptococcus antigen			
AFB cultures			
PPD			
Fungal culture			

*All infectious disease testing was negative except for the aerobic blood cultures. After 4 days of incubation, small Gram-negative coccobacilli were observed on a Gram stain prepared from the aerobic blood culture.

Polymerase chain reaction (PCR), Herpes simplex virus (HSV), Varicella zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Human herpesvirus-6 (HHV-6), Human immunodeficiency virus- 1 (HIV-1), Human immunodeficiency virus-2 (HIV-2), Acid-fast bacilli (AFB), Purified protein derivative (PPD), Enzyme immunoassay (EIA).

These microorganisms are able to persist by selectively invading human macrophages, where they can survive and evade the innate immune response of the host. This is first accomplished by evading toll-like receptors, then by preventing macrophage self-induced apoptosis. The mechanism of evasion of toll-like receptors is attributed to the lipid A component of the lipopolysaccharide present on *Brucella* spp. cell membranes. *Brucella* spp. modifies this lipid A so that toll-like receptors on the host macrophages cannot recognize them as Gram-negative bacteria, thus postponing activation and secretion of pro-inflammatory cytokines.^{3,6,11} The lipopolysaccharide also protects the bacterium by preventing the activation of the complement cascade system by inhibiting binding of complement protein C3. This inhibits production of C3a and C5a, which are pro-inflammatory proteins that also stimulate toll-like receptors.^{3,6}

The prevention of host cell apoptosis is attributed to the inhibited release of the pro-apoptotic and pro-inflammatory cytokines interferon- γ and TNF- α . TNF- α is inhibited via a membrane protein on *Brucella* spp., which in turn inhibits the recruitment of CD4+, CD8+, and natural killer cells. This

inhibition of cellular recruitment postpones the release of interferon- γ , preventing signaling of apoptosis in infected macrophages. Prevention of apoptosis enables intracellular proliferation and consequently prolongs the activation of macrophages and cells of the innate immune response. It is hypothesized that the balance of these cytokines mediates the outcome of cases of brucellosis. In this case, the balance was weighted in favor of the persistence of systemic infection.^{3,6,11}

Once *Brucella melitensis* has invaded host macrophages, these infected cells are disseminated throughout the body via hematogenous spread. The infected macrophages infiltrate various areas of the body with preference for the mononuclear phagocytic system. Common sites of involvement include the liver, spleen, bone marrow, and lymph nodes.³ The prolonged activation and systemic distribution of infected macrophages exacerbates the inflammatory state, which may trigger onset of HLH.

HLH is a rare, life-threatening syndrome characterized by a severe and prolonged hyper-inflammatory state caused by the inability of the immune response

to shut down in a normal manner.^{1,2,14} HLH may be inherited or it can be acquired through a strong immune activating event including malignancy, infectious agents, (particularly herpes viruses), toxins, or rheumatic diseases. It may also be acquired by endogenous means, such as metabolic products, extreme stress, or tissue damage.^{1,2,14} HLH can occur in all age groups, however, the inherited form is more commonly seen in children.¹⁵

Under normal circumstances, exposure to an antigen causes T-helper-1 cells to release cytokines, particularly TNF- α , interferon- γ , and monocyte colony stimulating factor. This causes activation of cytotoxic T-cells, natural killer cells, and macrophages, allowing for the removal of the antigen and therefore a cessation of the inflammatory response. In HLH, there is a defect in the cytotoxic pathway, causing persistence of the antigen, reduced apoptosis, and continued release of inflammatory cytokines and macrophage activation.¹⁵ The prolonged activation of macrophages and increased TNF- α and interferon- γ trigger phagocytosis of surrounding RBCs, platelets, and WBCs. Hemophagocytosis may occur in the bone marrow, liver, spleen and/or lymph nodes, and these hemophagocytic cells may persist and infiltrate other areas of the body.^{1,2,3} This infiltration can cause liver failure, bone marrow stress, and neurological abnormalities, which can be life threatening if left untreated.

Genetic HLH is caused by mutations of genes involved in the perforin protein pathway in cytotoxic cells. The mechanism by which infectious agents cause a defect with cytotoxic pathways and initiate HLH is not well understood.¹⁵ Epstein-Barr virus induced HLH has been well studied. In the cases that HLH occurred as a result of Epstein-Barr virus, infection of cytotoxic T-cells leads to impairment of the cytotoxic pathway that is characteristic of HLH.¹⁵

Manifestations of HLH may mimic other disease processes and differential diagnosis should include conditions such as malignant diseases, infections, systemic inflammatory response syndrome (SIRS), Langerhans cell histiocytosis, and macrophage activation syndrome (MAS). Cytopenia, markedly increased ferritin, and altered liver function help to

discriminate between HLH and other conditions. Infectious causes of acquired HLH must be investigated.^{14,15}

In cases of suspected HLH, laboratory testing should include CBC with differential, liver function tests, triglycerides, serum ferritin, and coagulation studies with fibrinogen, and bone marrow and cerebrospinal fluid analysis. Bone marrow biopsies may initially be negative for hemophagocytosis, so a repeat biopsy may need to be performed. Blood and urine specimens should be collected for infectious disease testing.^{1,16,15}

Treatment of HLH consists of chemotherapy, immunosuppressants, and corticosteroids to control the secretion of cytokines and activation of macrophages, which intensify the inflammatory state.^{1,3,16} In cases of acquired HLH, treatment of the primary cause of the prolonged inflammatory state will likely resolve the symptoms of HLH.¹⁶

Epidemiological data for acquired HLH is not well documented in the United States and is possibly underreported due to the invasive nature of bone marrow sampling, as well as difficulty in identifying hemophagocytosis.^{2,16}

CASE CONCLUSION

After administration of the quadruple neurobrucellosis therapy, the patient began to show signs of improvement. Shortly thereafter, HLH was resolved and the patient was started on an outpatient treatment plan of oral doxycycline, gentamicin, and rifampin with recommended monthly laboratory testing to monitor progress. When diagnosing acquired HLH, brucellosis should be considered when ruling out possible causes, particularly when the patient is from or has traveled to endemic areas.

ACKNOWLEDGEMENTS

A special thanks to the Department of Laboratory Medicine, Boston Children's Hospital for their assistance with this case.

REFERENCES

1. Janka G. Hemophagocytic syndromes. *Blood Rev.* 2007;21: 245-53.
2. Janka G, Zur Stadt U. Familial and acquired hemophagocytic lymphohistiocytosis. *ASH Education Program Book.*

CLINICAL PRACTICE

- 2005;1:82-8.
3. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. *N Engl J Med*. 2005;352:2325-36.
 4. Gul HC, Erdem H, Bek S. Overview of neurobrucellosis: a pooled analysis of 187 cases. *Int. J Infect Dis*. 2009;13:e339-43.
 5. Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al. *Koneman's color atlas and textbook of diagnostic microbiology*. 6th ed. Baltimore: Lippincott Williams & Wilkins; 2006.
 6. Atluri V, Xavier M, Jong M, Hartigh A, Tsois R. Interactions of the human pathogenic *Brucella* species with their hosts. *Annu Rev Microbiol* 2011;65:523-41. doi: 10.1146/annurev-micro-090110-102905
 7. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos E. The new global map of human brucellosis. *Lancet Infect Dis*. 2006;6:91-9.
 8. Fosgate GT, Carpenter TE, Chomel BB, Case JT, DeBess EE, Reilly KF. Time-space clustering of human brucellosis, California, 1973-1992. *Emerg Infect Dis*. 2002;8(7):672-8.
 9. Perkins P, Rogers A, Key M, Pappas V, Wende R, Epstein J, et al. Epidemiologic notes and reports brucellosis-Texas. *MMWR*. 1983;32(42):548-53.
 10. Willey JM, Sherwood LM, Woolverton CJ. *Prescott's microbiology*. 8th ed. New York: McGraw-Hill; 2011.
 11. Dornand J, Gross A, Lafont V, Liautard J, Oliaro J, Liautard JP. The innate immune response against brucella in humans. *Vet Microbiol*. 2002;90:383-94.
 12. cdc.gov[Internet]. Brucellosis- overview of laboratory risks. [updated 2012 November 12; cited 2015 August 3]. Available from: <http://www.cdc.gov/brucellosis/laboratories/risks.html>.
 13. Sari I, Altuntas F, Hacıoglu S, Kocyyigit I, Sevinc A, Sacar S, et al. A multicenter retrospective study defining the clinical and hematological manifestations of brucellosis and pancytopenia in a large series: hematological malignancies, the unusual cause of pancytopenia in patients with brucellosis. *Am J of Hematol*. 2008;83:334-9.
 14. Filipovich A. Hemophagocytic lymphohistiocytosis (HLH) and related disorders. *American Society of Hematology Education Book*. 2009 January; 1:127-131.
 15. Rosado FG, Kim AS. Hemophagocytic lymphohistiocytosis: an update on diagnosis and pathogenesis. *Am J Clin Pathol*. 2013;139:713-727. doi: 10.1309/AJCP4ZDKJ4ICOUAT
 16. Fisman DN. Hemophagocytic syndromes and infection. *Emerg Infect Dis*. 2000;6(6):601-8. doi: 10.3201/eid0606.000608.