A CASE REPORT CONCERNING THE EXPOSURE TO BRUCELLA SPP. OF A VETERINARY LABORATORY STAFF

UN RAPORT DE CAZ PRIVIND EXPUNEREA LA BRUCELOZĂ A PERSONALULUI UNUI LABORATOR VETERINAR

Elena AWWAD[1),2)], M. FARAJ[3)], T. ESSAWI[3)], K. ADWAN[4)], A. MANASRA[2)], M.V. CÎMPEANU[1)], Maria Rodica GURĂU[1)], D. COBZARIU[1)], S. BĂRĂITĂREANU[1)]

ABSTRACT | REZUMAT

The risk of laboratory-acquired brucellosis is increased in areas where brucellosis is still endemic. Brucella specimen is highly contagious when handled in laboratory. Despite the enforcement of infection control measures including the use of the biological safety cabinet, brucellosis is still very dangerous when the structure of the whole laboratory do not met the security requirements. Main reason of laboratory-acquired brucellosis in these reported cases was the inappropriate working flow design of the veterinary laboratory. The unit for samples reception is not properly located in the workflow and the implemented workflow ignores the right sense of the airflow: the open doors allow airflow to cross over all sections. In this report, we present three cases of laboratory-acquired brucellosis in veterinary laboratory. Based on the results of this study, the bacteriological investigations, mainly Brucella spp. cultures, should be performed only after refurbishment or reconstruction of the veterinary laboratory according to OIE, WHO and ISO requirements. Palestinian authorities, mainly the Ministry of Agriculture, should invest more resources for the infrastructure of the veterinary services and should provide adequate resources in order to enhance the requested capability and capacity of the veterinary laboratory to cover the disease surveillance needs and also the field investigations. Therefore, reducing of the occupational brucellosis and/or laboratory-acquired rely on the improvement of the facilities dedicated to the veterinary services in Palestine and the decrease of the brucellosis prevalence.

Keywords: bacterial diseases, brucellosis, zoonosis, laboratory contamination

Riscul de bruceloză dobândită în laborator este crescut în zonele în care aceasta este încă endemică. Probele de *Brucella* sunt foarte contagioase chiar și când sunt manipulate în condiții de laborator. În ciuda aplicării măsurilor de control, inclusiv a utilizării hotelor cu flux laminar, bruceloza este încă foarte periculoasă atunci când infrastructura laboratorului nu întrunește cerințele de securitate. În cazurile raportate, cauza principală a brucelozei dobândite în laborator a fost proiectarea inadecvată a fluxului de lucru în spațiile disponibile. Unitatea de recepție a probelor nu este localizată în mod corespunzător în cadrul instituției și fluxul de lucru implementat ignoră sensul corect al ventilației: ușile deschise permit fluxului de aer să traverseze toate încăperile laboratorului. Sunt prezentate trei cazuri de bruceloză dobândite în laboratorul veterinar. În urma acestui studiu, s-a recomandat efectuarea examenelor bacteriologice, mai ales cultivarea *Brucella* spp, numai după renovarea sau reconstrucția laboratorului veterinar în conformitate cu cerințele OIE, OMS și ISO. Autoritățile palestiniene, în principal Ministerul Agriculturii, ar trebui să investească mai multe resurse pentru infrastructura serviciilor veterinare și ar trebui să asigure resurse adecvate pentru a spori capabilitatea și capacitatea solicitată laboratorului veterinar pentru a acoperi nevoile de supraveghere a bolilor și investigațiile de teren. Prin urmare, reducerea bolilor profesionale de laborator în Palestina, se bazează pe îmbunătățirea infrastructurii dedicate serviciilor veterinare și pe scăderea prevalenței brucelozei în exploatații.

Cuvinte cheie: boli bacteriene, bruceloză, zoonoză, contaminarea de laborator

1) University of Agronomic Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania
2) Ministry of Agriculture, General Directorate of Veterinary Services and Animal Health, Central Veterinary Laboratory, Ramallah, Palestine
3) Master Program in Clinical Laboratory Science (MCLS), Birzeit University, Birzeit, Palestine
4) Department of Biology and Biotechnology, An-Najah National University, Nablus, Palestine
First author e-mail: eleenaawwad@yahoo.com
Corresponding author e-mail: stelianbaraitareanu@fmvb.ro

Brucellosis is an important zoonotic disease worldwide and for the endemic areas is placed in top of the list [4, 7]. The *Brucella melitensis* infections in small ruminants are still widespread in the Mediterranean region and the human illness is usually reported.

Human contamination is primarily by consumption of contaminated dairy products or by occupational exposure to infected livestock [4, 8, 14, 18].

In humans, if the disease is not diagnosed and treated promptly and effectively, it can become chronic and affect multiple body systems [5, 14, 18, 19]. In laboratories, *Brucella* spp are one of most frequently acquired infections [15]. Microbiology laboratory workers have been identified as being at increased risk of brucellosis through unsuspected exposure to infected specimens and their cultures. Laboratory-acquired infections represent 2% of all brucellosis cases worldwide [17, 21, 22].Transmission usually occurs through inhalation of pathogen when making bacterial suspension and pipetting, when working on an open bench with viable organisms [3, 15, 21], or when the safety cabinet ventilation system failed [13].

The organism may enter the body in many ways related to laboratory practices (e.g., through the respiratory mucosa, conjunctivae, gastrointestinal tract, or through the skin with or without wounds) [22].

Soon after the entry into the body, *Brucella* is ingested by polymorphonuclear and mononuclear phagocytes. The organism is able to escape phagocytic lisis by inhibiting the phagosome-lysosome fusion, is multiplying inside macrophages and is disseminating though the body [23]. Most accidents of laboratory-acquired brucellosis have been caused by *Brucella melitensis* [1, 3, 9, 16, 17, 20, 21], but there are also laboratory human infections caused by *Brucella abortus* [6], *Brucella canis* [2], or even by vaccine strains, *Brucella melitensis* Rev 1 and *Brucella abortus* S19 [3]. The clinical pattern of the brucellosis in human is nonspecific, the systemic symptoms as fever, headache, malaise are associated with night sweats and arthralgia, resembling a flu-like syndrome [4,14,18,19].

The primary goal of this study is to evaluate the risk of human contamination when the preventive medicine policy is neglected, based on the human cases reported in the Palestinian Central Veterinary Laboratory. No previously articles have been published, this paper being the first report of brucellosis outbreaks in Palestinian Central Veterinary Laboratory.

MATERIALS AND METHODS

Case reports.

Case report 1: November 2012, 44-years old woman in charge with the isolation of *Brucella* has presented undulant prolonged fever with body temperature 38.7°C, ataxia, anorexia, sleep hyperhidrosis, headache, and joints pain. All hematological and routine biochemical parameters were in normal limits, erythrocyte sedimentation rate (ESR) was 68 mm/h. Rose Bengal test (RBT) showed +4 positive for *Brucella* antigen, serum agglutination test (SAT) titer was reactive 1:320 and positive for ELISA *Brucella* IgM (strong) and IgG (weak) [11].

Case report 2: November 2012, one week after the first case report, 43-years old man, in charge with PCR analysis,had fever,body temperature 38°C, ataxia, and sleep hyperhidrosis. The hemogram and routine biochemical test were within normal range, but ESR was 40 pm/h, RBT +3 positive, and SAT 1:160 [11].

Case report 3: November 2012, one week after the second reported case, 37-years old man, in charge with documentation and checkup of laboratory investigations had similar signs, RBT showed +3, and SAT 1:80 [11].

The classical treatment, rifampicin (600 mg) and doxycycline (100 mg) for 30 days was set. The clinical status has recovered in the first few days of treatment, although tiredness persisted. When treatment was finished, RBT show weak titer (±1). Six month later, similar signs were repeated and all laboratory tests for *Brucella* were positive in the first two cases. The first case has relapsed with ocular complication and the laboratory tests, RBT, SAT and ELISA IgM, were strong positive, but ELISA IgG was mild positive [11]. Therapy has been repeated for the cases 1 and 2, in addition with intravenous cortisone for the first one. No relapse occurs in the third case. All infected persons have no history of eating unpasteurized dairy product and contact with any infected animals.

Infrastructure of the Palestinian Veterinary Laboratory

Palestine is an endemic brucellosis area and, since 1998, the isolation of *Brucella* spp. in the Central Veterinary Laboratory is a routine procedure, using specimens of milk and aborted fetus. Isolation of *Brucella* or other pathogens was always performed in class II biological safety cabinet by the staff trained to handle biohazardous agents; no cases of *Brucella* infection among laboratory staff occur before 2012.

Specifications of the laboratory facilities in Ramallah had fulfilled the biosafety requirements, but it was under capacity and unsuitable for processing large numbers of samples. In 2011, the laboratory was relocated in Aroub, under the policy maker authority [12]. The new location does not fulfill the biosafety requirements of the veterinary laboratory, the building being designed to be laboratory for testing the olive oil.

This building has a big door (3 m), always open into the common corridor, and there is no interlocking

door system between the laboratories rooms. The desk for samples reception and registration was located in the middle of the main corridor, between the laboratory rooms.

RESULTS AND DISCUSSION

Laboratory staff involved directly in *Brucella* bacteriology (e.g. bacteriologists, laboratory assistants) is more frequently exposed than others (e.g., researchers). Unfortunately, the moment of accidental exposure is often unknown and the suspicion of infection occurs at the onset of illness [17].

In developing countries, the risk of the professional exposure is frequently aggravated by lack of suitable protective equipment and inadequate laboratory facilities [3].

In the broader context of biosecurity, the investigation of the brucellosis cases laboratory-acquired suggest that the critical risk factor of infection has been the manipulation of Brucella suspected specimens into the inadequate reception area and the airborne dissemination of the pathogen over all sections of laboratory through the uncontrolled airflow. Time of infection is estimated to seven weeks before the disease onset. The isolation of bacteria and enzyme-based tests for the identification were carried out in class II biological safety cabinet by microbiologists trained to handle biohazardous agents. Memish et al. (2001) reported that brucellosis risk is still high among laboratory workers despite the implemented precautions as the use of safety cabinets. They related this result to the large number of brucellosis suspected materials sent to the laboratory [10]. Main reason of brucellosis cases in Palestinian Central Veterinary Laboratory was related to the transfer of the laboratory into an inadequate and unsafe infrastructure: the reception unit of samples is not properly located in the workflow and the implemented workflow ignores the correct sense of the airflow which caused the spread of pathogen through all area.

Another risk of Brucella spreading has been the handling of inoculated media during additional investigations: staining of smears was doing on staining jar and the stain was collected on jar; but it does not explain the case of brucellosis in non-microbiology section workers. As result of this cases occurrence, isolation of bacteria was temporally stopped and there were improved the biosafety measures of handling pathological material. It immediately started the design plan for the refurbishment of the laboratory, according to OIE, WHO and ISO requirements. In 2015, it started renovation of the laboratory.

It should be emphasize that brucellosis could be transmitted in laboratory and, in order to avoid the pathogen dissemination and to prevent the contamination of staff, must be implemented good laboratory practice (GLP) rules and CDC recommendations. Each member of the laboratory staff must be motivated to work cautiously and responsible toward laboratory risks; handling of infectious material must be carried out into the level 3 biosafety cabinet. These should comply the standards for the infrastructures dedicated to the veterinary services and should also comply with sustainable policy. Unfortunately, the stability of the structure of the veterinary services and the political *status* did not allow to design and implement long-term strategies and appropriate policies for improving veterinary services in Palestine.

CONCLUSIONS

Brucellosis remains a significant problem for the animal and human health in Palestine, despite on the intensive vaccination programs. To face this challenge, it is necessary to build a new veterinary laboratory, in the central area of the country, outside of the city, which complies with all national and international standards of biosecurity. Reducing the occupational brucellosis and/or laboratory-acquired rely on the improvement of the facilities dedicated to the veterinary services in Palestine and the decrease of the brucellosis prevalence.

REFERENCES

1. *Batchelor B., Brindle R., Gilks G., Selkon J.,* (1992), Biochemical misidentification of Brucella melitensis and subsequent laboratory-acquired infections, J Hosp Infect 22:159-162.
2. *Blankenship R., Sanford J.,* (1975), Brucella canis. A cause of undulant fever, Am J Med 59:424-426.
3. *Corbel M.,* (2006), Brucellosis in human and animals. Produced by: FAO, OIE, WHO organizations.
4. *Doganay M., Aygen B.,* (2003), Human brucellosis: an overview, Int J Infect Dis 7:173-182.
5. *FAO,* (2010), Brucella melitensis in Eurasia and the Middle East. FAO Animal Production and Health Proceedings, No. 10, Rome.
6. *Fiori P., Mastrandrea S., Rappelli P, Cappuccinelli P,* (2000),Brucella abortus infection acquired in microbiology laboratories, J Clin Microbiol 38:2005-2006.

7. *Gul T., Khan A.,* (2007), Epidemiology and epizootology of brucellosis: A review, Pakistan Veterinary Journal 27:145−151.
8. *Kaoud A., Zaki M., El-Dahshan A., Nasr S.,* (2010), Epidemiology of brucellosis among farm animals, Nature and Science 8:190-197.
9. *Martin-Mazuelos E., Nogales M., Florez C., Gomez-Mateos J., Lozano F., Sanchez A.,* (1994), Outbreak of Brucella melitensis among microbiology laboratory workers, J Clin Microb, 32:2035-2036.
10. *Memish Z., Mah M.,* (2001), Brucellosis in laboratory workers at a Saudi Arabia hospital, Am J Infect Control 29:48.
11. *Ministry of Health,* (2012), Personal laboratory report, Archive of Ministry of Health.
12. *Ministry of Agriculture,* (2011), Yearly report, Archive of Ministry of Agriculture.
13. *Rodrigues A., Silva S., Pinto B., Silva J., Tupinambás U.,* (2013), Outbreak of laboratory-acquired Brucella abortus in Brazil: a case report, Revista da Sociedade Brasileira de Medicina Tropical 46(6): 791-794.
14. *Seleem N., Boyle M., Sriranganathan N.,* (2010), Brucellosis: a reemerging zoonosis, Veterinary Microbiology, 140(3-4):392–398.
15. *Singh K.,* (2009), Laboratory-Acquired Infections, Clinical Infectious Diseases 49:142–147.
16. *Staszkiewicz J., Lewis C., Colville J., Zervos M., Band J.,* (1991), Outbreak of Brucella melitensis among microbiology laboratory workers in a community hospital, J Clin Microbiol 29:287-290.
17. *Traxler R., Lehman M., Bosserman E., Guerra M., Smith T.,* (2013), A Literature Review of Laboratory-Acquired Brucellosis, Journal of Clinical Microbiology 51(9):3055-3062.
18. *Zvizdic S., Cengic D., Bratic M., Mehanic S., Pinjo F., Hamzic S.,* (2006), Brucella melitensis review of the human infection case, Bosnian Journal of Basic Medical Sciences 6:15-18.
19. *World Health Organization,* (1999), Human and animal brucellosis: Epidemiological Surveillance in the MZCP Countries, Report of a WHO/MZCP Workshop, Damascus, Syrian Arab Requblic, 4-5 May 1998. Athens 1999.
20. *Yagupsky P., Baron E.,* (2005), Laboratory Exposures to Brucellae and Implications for Bioterrorism, Emerg Infect Dis 11:1180-1185.
21. *Yagupsky P., Peled N., Riesenberg K., Banal M.,* (2000), Exposure of hospital personnel to Brucella melitensis and occurrence of laboratory-acquired disease in endemic area, Scand J Inf Dis 32:31-35.
22. *Young E.,* (2005), Brucella species. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's principles and practice of infectious diseases. Philadelphia: Elsevier, Churchill, Livingstone; p. 2669-2674.
23. *Xavier M., Paixao T., Hartigh A., Tsolis R., Santos R,* (2010), Pathogenesis of Brucella spp., The Open Veterinary Science Journal, 2010, 4, 109-118.