Case Report

The first report of *Brucella melitensis* Rev.1 human brucellosis in Bosnia and Herzegovina

Jurica Arapović¹,², Silvio Špičić³, Sanja Duvnjak³, Maja Ostojić⁴, Maja Arapović⁵, Jadranka Nikolić¹,², Željko Cvetnić³

¹ Department of Infectious Diseases, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina
² Faculty of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina
³ Laboratory for Bacterial Zoonosis and Molecular Diagnostics of Bacterial Diseases, Croatian Veterinary Institute, Zagreb, Croatia
⁴ Institute for Microbiology and Molecular Diagnostics, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina
⁵ Veterinary Institute of Herzegovina-Neretva Canton, Mostar, Bosnia and Herzegovina

Abstract

Brucellosis is an emergent and endemic zoonotic disease in Bosnia and Herzegovina. In this report we have diagnosed the first case of human brucellosis in Bosnia and Herzegovina, using molecular and microbiological tests, caused by live attenuated *Brucella melitensis* Rev.1 strain. The infection was caused through unintentional exposure to vaccination of small ruminants in Bosnia and Herzegovina and without any prior accidental self-injection of vaccine suspension.

Key words: Bosnia and Herzegovina; *Brucella melitensis*; brucellosis; Rev.1 vaccine.


(Received 21 August 2019 – Accepted 14 January 2020)

Copyright © 2020 Arapović et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

A live attenuated *Brucella melitensis* Rev.1 strain is systematically used for the vaccination of small ruminants worldwide. Brucellosis is a zoonosis so this action not only prevents its spreading among small ruminants and other animals but also to humans [1]. The source of human infection is always in domestic or wild animal reservoirs and as a general rule, human brucellosis prevention depends on the control of this zoonotic pathogen in animals [2,3].

It has been previously suggested that the Rev.1 vaccine strain does not cause disease in non-pregnant ruminants and that it is not pathogenic for other animal experimental models [4,5]. However, experimental models of intradermal or subcutaneous application in humans clearly showed that vaccinal Rev.1 strain cannot be used for immunization of humans. Also, it has been suggested that this strain can cause acute brucellosis in vaccinated individuals; only very small inoculum of Rev.1 vaccine failed to cause disease [6,7]. To date, there is no universal vaccine for humans and pigs [8].

Nevertheless, the virulence of the Rev.1 strain for humans is still uncertain, since a small number of publications support possible transmission of Rev.1 strain to humans. They argue that the infection is only possible after accidental inoculation and that aerogenic transmission or other contact is only possible under very specific conditions [9-13].

In this report, we describe the first case of human Rev.1 brucellosis in Bosnia and Herzegovina in a patient who was involved in vaccination of small ruminants. It is important to emphasize that there was no known incident of self-injection of the vaccine in this case.

Case Report

A 48-year old man was hospitalized because of fever, chills, fatigue, night sweats and back pain which lasted for 10 days prior to the admission. He was diagnosed with type II diabetes and gastritis and started using metformin 500 mg twice a day (BID) and pantoprazole 40 mg BID per os (p.o.) three months ago.

Four days prior to hospitalization, he had visited a physician who prescribed cefixime 400 mg once a day...
Table 1. The MLVA-16 of human Rev.1 isolate compared to other strains isolated in BH.

<table>
<thead>
<tr>
<th>No.</th>
<th>SPECIES ORIGIN YEAR HOST</th>
<th>locus 06</th>
<th>locus 08</th>
<th>locus 11</th>
<th>locus 12</th>
<th>locus 14</th>
<th>locus 15</th>
<th>locus 18</th>
<th>locus 21</th>
<th>locus 04</th>
<th>locus 07</th>
<th>locus 16</th>
<th>locus 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B. melitensis Rev.1 UN</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>8</td>
<td>36</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>B. melitensis Rev.1 BH</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>36</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>B. melitensis bv. 1 Sweden</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>36</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>B. melitensis bv. 3 BH</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>40</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>B. melitensis Rev.1 BH</td>
<td>2016</td>
<td>human</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>B. melitensis bs. 3 BH</td>
<td>2016</td>
<td>human</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>B. melitensis bs. 3 BH</td>
<td>2016</td>
<td>human</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>B. melitensis bs. 3 BH</td>
<td>2016</td>
<td>human</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>9</td>
<td>B. melitensis bs. 3 BH</td>
<td>2016</td>
<td>human</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>40</td>
</tr>
</tbody>
</table>

*No, Number of isolate; UN, undefined; BH, Bosnia and Herzegovina.

Table 2. Vaccine strains with identical MLVA-16 genotype in Brucella MLVA database.

<table>
<thead>
<tr>
<th>SPECIES ORIGIN YEAR HOST</th>
<th>locus 06</th>
<th>locus 08</th>
<th>locus 11</th>
<th>locus 12</th>
<th>locus 14</th>
<th>locus 15</th>
<th>locus 18</th>
<th>locus 21</th>
<th>locus 04</th>
<th>locus 07</th>
<th>locus 16</th>
<th>locus 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. melitensis bv. 1 Italy 2011 goat</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>36</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>B. melitensis bv. 1 Israel 1996 sheep</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>36</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>B. melitensis Rev.1 Portugal 2006 UN</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>36</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>B. melitensis Rev.1 Portugal 2006 UN</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>36</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>B. melitensis Rev.1 BH 2016 human</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>36</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

*UN-undefined; BH-Bosnia and Herzegovina.

and metronidazole 400 mg three times a day for the last two days, assuming that the origin of infection could be of urogenital or gastrointestinal etiology. Since the patient had nausea and continuous hiccups, a gastroscopy was also performed. Hiatal hernia was observed without any other pathomorphological finding.

Significant epidemiological findings were the continuous contact with sheep and interaction with his own small herd of sheep. The epidemiological history revealed that he was also involved in vaccination of his friend’s sheep in the southern part of Bosnia and Herzegovina. He did not wear any protective clothing such as a suit, or gloves, glasses and mask while handling the animals, whereas the veterinarian who performed the vaccination wore only gloves.

At admission to hospital, the patient was febrile (axillary body temperature, T<sub>ax</sub> 40.3°C) with pronounced algic syndrome. The blood pressure was within normal range (RR 115/70 mm Hg), heart rate was 110/min, and respiratory frequency was normal (18/min). There was no evidence of lumbago at that moment and all other physical examination findings were normal, without neurological abnormalities. Laboratory findings were as follows: white blood cell (WBC) count 3.2×10<sup>9</sup>/L (neutrophils 50%, lymphocytes 45%, basophiles 1%, monocytes 4%), erythrocyte blood cell (RBC) count 4.86×10<sup>12</sup>/L, hemoglobin 148 g/L, thrombocytes 120×10<sup>9</sup>/L, C-reactive protein 16.5 mg/L, erythrocyte sedimentation rate (ESR) 13 mm/hour, blood glucose 6.5 mmol/L, bilirubin 22.1 µmol/L, lactate dehydrogenase 397 mmol/L, creatinine 140 mmol/L, creatine kinase 444 U/L. Results of all other laboratory findings including urine were in normal range.

Chest radiography was normal, whereas abdominal ultrasound showed splenomegaly (16.8 cm) and hepatomegaly (18.5 cm measured in medio-clavicular line).

Serological testing for brucellosis using the Rose-Bengal slide agglutination test was positive and Brucellae were isolated from four (two sets – aerobic and anaerobic) independently-taken blood cultures. The antibiogram was not done routinely and isolates were stored in SkimMilk® media at -20°C until molecular diagnostics were performed. Molecular identification was done later using the Bruce-ladder method [14]. The Bruce-ladder test is a multiplex polymerase chain reaction test (PCR) that enables a single step identification of the following Brucella species: all terrestrial Brucella species (B. neotomae; B. microti, B. inopinata; B. abortus - biosvars 1, 2, 3, 4, 5, 6, 7, 9; B. melitensis biosvars 1, 2, 3 and B. suis biosvars 1, 2, 3, 4, 5), strains isolated primarily from marine mammals (B. pinnipedialis and B. ceti) and vaccine strains (B. abortus S19, B. abortus RB51 and B. melitensis Rev.1). This is possible through detection of presence or absence of amplification using 8 different primer pairs, giving a final profile specific for a given species. B. melitensis Rev.1 strain was determined. Additionally, the strain was genotyped by Multi-Locus Variable Number Tandem Repeat Analysis on 16 loci (MLVA-16) [15,16]. MLVA-16 results were presented in the form of a genotype: a 16-digit numerical code.
comprised of 16 alleles defined on 16 different loci. The genotype was compared to genotypes of strains isolated from human brucellosis cases diagnosed in the same year in our hospital [17]. The genotype of our human Rev.1 isolate was identical to that of a vaccinal strain from a sheep in 2010 in Bosnia and Herzegovina (Table 1). The strains were also compared to strains present in Brucella MLVA database version 3.6 (http://mlva.u-psud.fr) [18]. Identical genotypes were also identified in four strains isolated from ruminants in Italy, Israel and Portugal (Table 2).

The therapeutic regime was started with doxycycline 100 mg p.o. twice daily (BID) and gentamicin 5 mg/kg divided in two doses intravenously (i.v.). He was febrile for four days and became afebrile afterwards, while his general condition improved. After 21 days of treatment at the hospital, the patient was discharged. Antimicrobial therapy was continued at home with doxycycline (100 mg p.o. BID) and rifampicin (300 mg p.o. BID) for a total of three months.

Discussion

Brucellosis is an emergent and endemic zoonotic disease in Bosnia and Herzegovina and to date responsible for more than 3,000 patients recorded in the Federation of Bosnia and Herzegovina. In the Federation of Bosnia and Herzegovina, the highest incidence occurred in 2008 with 33.4 per 100,000 inhabitants and with 778 registered patients [19, 20].

The systemic vaccination program of small ruminants via the conjunctival route has been implemented in the Federation of Bosnia and Herzegovina from June 1st, 2009 [19]. A few years later, in 2012, the incidence of human brucellosis rapidly decreased, more than 10 fold, down to 59 registered patients with an incidence of 2.52 per 100,000 inhabitants [20].

The patient presented in this report was involved in the vaccination process as an assistant to the veterinarian who performed vaccination of sheep via conjunctival route. Based on the deep epidemiological examination, we assumed that he was probably infected via conjunctival or aerogenic transmission, since no local lymphadenopathy or skin injury was noticed as a possible entry site of infection.

Ashford et al. clearly showed the possibility of infections by the live attenuated B. abortus vaccinal strain RB51 after an accidental inoculation or conjunctival splashing [21]. According to the intrinsic resistance of Rev.1 strain to streptomycin [5], the therapeutic regime should be started with combination of doxycycline and rifampicin for at least six weeks [10-12]. An antibiogram was not performed for the patient presented in this report, and the therapy was started with a standard combination of doxycycline 100 mg BID p.o. and gentamicin 5 mg/kg divided in two doses per day i.v. This confirms the importance of an antibiogram during an epidemic of brucellosis, especially in a region where a massive ruminant vaccination program with the Rev.1 strain had been implemented.

The crucial point of this report is that wearing recommended clothing should improve protection of staff performing vaccination [13,22]. This patient did not wear recommended protection or take any antimicrobial post-exposure prophylaxis, although it is recommended by both WHO and CDC [22].

Furthermore, it was previously demonstrated that different populations of T cells predominantly control brucellosis in the host [23]. Such immunity could also be affected in some chronic diseases such as diabetes [24]. Taking this into account, there is a possibility that diabetes in this patient was an additional risk factor for the manifestation of disease after transmission of small inoculum of Rev.1 strain. Similar conditions have been previously observed [25].

At the moment, no routine serological tests exist which can differentiate between animals vaccinated with Rev.1 and animals infected with virulent wild type (w.t.) Brucella species [2,26]. Thus, blood culture isolation is still the “gold” standard for determination of Brucella species in humans [2,8]. However, at least two molecular methods can differentiate between w.t. B. melitensis and Rev.1 vaccinal strain [9,14]. Here we showed that in addition to Bruce-ladder, Rev.1 human isolate could also be identified by the MLVA-16 assay showing that the genotypes were identical [27].

In conclusion, this report demonstrated the consequences of occupational exposure to B. melitensis vaccine Rev.1 strain. It emphasizes the need for surveillance of unintentional human exposure to Rev.1 and assesses its outcome. Prior to and during vaccination of small ruminants by Rev.1 vaccine, it is strongly recommended to wear protective clothes, glasses and mask. In the case that humans involved in vaccination were exposed to the vaccine without any of the recommended protection, post-exposure prophylaxis by doxycycline 100 mg p.o. BID and rifampicin 300 mg BID p.o. for three weeks should be taken. Also, identification of B. melitensis Rev.1 as a causative agent of infection in humans and animals should be a part of routine diagnostic procedure in endemic countries such as Bosnia and Herzegovina.
Acknowledgements
We would like to thank Dr. Željana Sulaver for critical reading of the manuscript.

Authors Contribution
JA coordinated all correspondence, designed and wrote the first version of the manuscript, and handled patients' approval for publication of personal data. JA, SS, MA, ŽC and JN contributed to the conception and writing of the first version of manuscript. JA, MO and JN contributed to clinical analysis. JA, SS, SD, MO, MA, ŽC and JN contributed to the data interpretation. JA, SS and SD contributed to the interpretation of figures. JA and JN conducted all epidemiological measures at the outbreak site. JA, MO, MA, SS, SD and ŽC provided isolation and molecular analysis of Rev.1 strain. All authors contributed to the writing of the final version of manuscript.

References

Corresponding author
Jurica Arapovic, MD, PhD, ID specialist
Associate Professor
Department of Infectious Diseases, University Clinical Hospital Mostar
Kralja Tvrtka b.b. 88000, Mostar, Bosnia and Herzegovina
Phone: +387 36 336 581; +387 36 336 577
Fax: +387 36 328 072
E-mail: jurica.arapovic@mef.sum.ba

Conflict of interests: No conflict of interests is declared.