



BRUCELLA MELITENSIS REVIEW OF THE HUMAN INFECTION CASE

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ABSTRACT

During the last several years, brucellosis has become an important public-health problem on a large territory part of Bosnia and Herzegovina. The disease belongs to the zoonosis group, and can be caused by several bacterium species from *Brucella* genus. For human and veterinarian medicine, *B. abortus*, *B. melitensis*, *B. suis* and *B. canis* from *Brucella* genus are important, while other brucella species are found only in animals. The results of laboratory process of isolating *Brucella melitensis*, as well as of detection of specific antibacterial antibodies, are presented in this work. Namely, *B. melitensis* was isolated from blood samples (chemo-culture), as a causal agent of disease in one sixty years-old patient, treated during 2001. In pair serum samples of the patient, the presence of specific anti-brucella antibodies was confirmed qualitatively and quantitatively. In the serum I, ELISA test confirmed the presence of specific IgM antibodies of 25,7 U/ml, and IgG antibodies of 252 U/ml. In the serum II, IgM antibodies of 24,9 U/ml, and IgG antibodies of 311 U/ml were found. These results suggest and confirm established work diagnosis, and etiology causality of the disease with isolated bacterium.

KEY WORDS: Brucellosis, *B. melitensis*, human infections, bacterium isolation, serology

INTRODUCTION

Brucellosis is one of infective diseases from the group of zoonoses and has characteristics of systemic disease. The infection is primary infective disease of domestic animals, from which it is transmitted to humans, directly or indirectly. Today, several brucella species are known, which infect and live as natural parasites of number of animal species. Four brucella species (*B. abortus*, *B. melitensis*, *B. suis* and *B. canis*) can infect animals and humans and cause brucellosis. These species of infec-

tive agents are transmitted from animals to humans. Inter-human infections are infrequent. *B. abortus* infects cattle, but it can also be isolated from horses, camels, buffalos and American bison. *B. melitensis* infects goats and sheep, while primary hosts for *B. suis* are pigs, reindeers, and elks, and dogs for *B. canis*. For humans, *B. ovis*, *B. neotomae*, and other species are not pathogenic (1,2,3,4,5). All brucellae are related to a lifelong chronic animal infection, since they are found within the cells of their milk glands and reproductive system. This is the way they can cause abortion or sterility in these animals. They are transmitted from infected to healthy animals. The infection is spreading via contaminated milk, urine, birth and abortion products, or aerosols (6). Pathogenic species of these bacteria are transmitted to humans by direct contact, through damaged skin or mucus, by consuming contaminated milk and milk products. Agricultural laborers, cattle breeders, veterinarians, and other personnel, who are constantly in the contact with animals, are more exposed to this infection, compared to other populations (5). Using unpasteurized milk and milk products increases the risk of brucellosis occurrence, but this is not the only route of a possible infection. The aerogene route of bacterium spreading is also important. Brucellosis is one of possible laboratory infections. The infection occurs as a result of working in unprotected environment, through skin, mouth, and eyes, not using gloves, mouth masks, protecting glasses, by mouth pipetting, which are the ways of exposing eyes, nose or mouth to contaminated aerosol (6). Brucellae are facultative intracellular parasites, which penetrate into the blood after entering a host. They disseminate from blood to different organs, usually locating themselves into the cells of reticuloendothelial system, and living inside phagocytes. Granulomas or abscesses can be formed on infection locus, which includes bone marrow, liver, spleen, lymph nodes, or lungs. Other infection loci can include subcutaneous tissue, testicles, ovaries, gallbladder, kidneys, and brain. Their infection is accompanied by development of meningitis and endocarditis. Brucellosis is systemic disease whose infective agents are found in any organ. Clinical manifestations of the disease can be different. The incubation lasts from 10 to 14 days, but it can vary for 5, and more than 35 days (1,4,5). The disease is acute, it occurs abruptly, and is characterized by high body temperature, night sweating, anorexia, headache, laxity of organism, and pains in different parts of the body. In subacute-chronic stage, there are intermittent temperature and local symptoms from individual organs, which are characteristics of the disease itself. This condition occurs in patients without a treatment, or

as the result of discontinuation of consuming medicines, which means that it develops as the result of using inadequate antibiotic therapy. This stage of the disease can last for months or years. Brucellosis treatment includes a long-term antibiotic therapy. Disease recurrences can occur 3 to 6 months after an early therapy discontinuation, or if bacteria are inapproachable for antibiotics. Chronic brucellosis is characterized by persistently high IgG antibody titers. Recommended antibiotic therapy includes a long-term usage of a doxycycline and streptomycine combination, along with symptomatic therapy (1, 4). The disease diagnosis is made by isolation of a causal agent, or in a patient's serum, by detection of specific antibodies using appropriate serology methods. Brucella can also be isolated from blood samples, bone marrow, liver, spleen, or abscess. For serology testing, more blood samples are necessary – chemo culture, in order to obtain optimal results during the febrile disease episodes. It is necessary to know that chemo cultures, as well as other tested materials, must be cultivated for a long time in optimal conditions and on adequate media. If it is possible, patient's material for cultivating should be collected before starting the antibiotic therapy. Blood is the best patient's material for successful brucella isolation. Serum samples from acute disease phase are collected immediately, while other and each next serum sample is collected after 14 to 21 days (1,3,4,5,6).

PATIENTS AND METHODS

This work describes the disease progress, therapy results, and microbiological identification of *B. melitensis* – of infected sixty years-old patient, treated during 2001 on the Clinic for Hematology and Clinic for Infective Diseases Clinical Centre Sarajevo. The blood was collected for cultivating (chemo culture) several times. Chemo cultures for aerobic and anaerobic cultivating had elongated incubation time, to one month. Positive chemo culture samples were further cultivated for isolation and final identification of causal agent. Microscopic, cultural, biochemical and serological testing and identification of causal agent were performed. In order to confirm obtained microbiology finding, the sample of positive chemo culture, as well as the serum of the patient, were sent to Croatian Veterinarian Institute, Zagreb (Republic of Croatia), where isolated bacteria were also identified. In the Institute, the presence of specific antibodies in the pair serum sample was confirmed using serology reactions of slow agglutination, Rose Bengal, and reaction of complement binding. In order to detect antibodies against *B. abortus*, *B. suis* and

B. melitensis, dead cells of *B. abortus* are used in the agglutination test. The pair serum sample of the patient was also sent to the WHO Reference Center for Arbovirus in Ljubljana (Slovenia), for qualitative and quantitative confirmation of the presence of specific anti-brucella antibodies, using the enzymatic method (ELISA).

RESULTS

The patient was hospitalized during the period from October 1st to December 7th 2001. The reasons for his hospitalization were a high body temperature, shuddering, pains in the muscles, weight loss, weakness, getting tired rapidly, the occurrence of spotty bleeding over the under-knee skin, as well as frequent urinating. According to the epidemiological questionnaire, the infected patient has been living in a suburban area, and he has owned a cow and a goat. During this period, other family members had no health difficulties. After the occurrence of these clinical symptoms, and because of his petechial bleeding, he was hospitalized to the Clinic for Hematology, where he was treated for 15 days, after which he was relocated to the Clinic for Infective Diseases Sarajevo. After admitting, the patient was hypo-dynamic, eupnaeic, dehydrating, had subicteric scleras, was sub-febrile, and had erythematous facial skin and significant spider nevi. There were middle abundant petechial bleedings on the under-knee skin. The lung finding indicated left basal reduced breathing. Objective cardiac finding indicated individual extra-systoles without heart murmurs, with clear heart tones (RR 110/60 mmHg). The liver was amplified for 2 to 2,5 cm, as well as the spleen for 0,5 cm. After admitting to the Clinic for Infective Diseases, the results of blood analyses showed the following values: SE-40/75, E-3,07, Hgb-85, HTC-0,25, L-2,9, Tr-40, ASAT-1905, ALAT-1763, gammaGT-2164 (Table 1). During the first days of hospitalization, intermittent temperature increased to 39°C, and hepatosplenomegaly was in progress. Ascites development was suspected,

along with the occurrence of new petechial bleedings. From the sixth day of antibiotic therapy, until releasing from the Clinic, the patient was afebrile, and since the 14th day of hospitalization there has been no new bleedings registered. The hepatosplenomegaly was in the satisfactory regress. While leaving the Clinic, the control results of individual blood parameters had the following values: SE-10/22, E-4,62, Hgb-124, HTC-0,41, L-4,2, Tr-101, ASAT-720, ALAT-690, gammaGT-1508 (Table 1). The blood was collected several times in order to cultivate it (chemo culture). After certain period, microbiology finding was obtained, and suggested that *Brucella sp.* was isolated from blood, and later classified as *B. melitensis*. Several samples of blood serum were also collected in a continuity, in order to confirm and observe the antibody titer. The agglutination test for brucellae gave a positive result, while specific IgM and IgG antibodies were detected by ELISA method. The results of ELISA test had the following values: serum I ELISA Brucella-IgM 25.7 U/ml (positive), ELISA Brucella-IgG 252 U/ml (positive), serum II ELISA Brucella-IgM 24.9 U/ml (positive), ELISA Brucella-IgG 311 U/ml (positive) (Table 2). These results confirm the work diagnosis previously established by doctors on the Clinic for Infective Diseases Sarajevo, as well as microbiological identification of disease's causal agent (*B. melitensis*). An adequate antibiotic therapy is started, which included the use of STM (streptomycine) ampullas *intramuscularly*, for three weeks, and doxycyclin pills, for six weeks. This therapy also included appropriate symptomatic and other necessary therapy. *B. Melitensis* is final identification finding.

DISCUSSION

Since brucellae are found in natural hosts in human's environment, from which they can be transmitted by particular routes and can cause human brucellosis, it is important, from epidemiological aspect, for them to be

HOSPITALIZATION PERIOD	SE	E	Hgb	HTC	L	Tr	ASAT	ALAT	gammaGT
ADMITTING	40/75	3,07	85	0,25	2,9	40	1905	1763	2164
RELEASING	10/22	4,62	124	0,41	4,2	101	720	690	1508

TABLE 1. The values of blood parametres during hospitalization

SERUM	Anti-Brucella IgM	Anti-Brucella IgG
THE FIRST SERUM	25.7 U/ml	252 U/ml
THE SECOND SERUM	24.9 U/ml	311 U/ml

TABLE 2. The results of serology diagnostic by ELISA test

detected and treated on time. *B. abortus*, which primary infects cattle (cows), is usually isolated from human material samples, while *B. melitensis* is isolated from goats and sheep, and rarely from human material. Controlling domestic animal disease, their constant serological controls, vaccination, and elimination of infected cattle, has reduced the number of infected animals in particular regions of the world. In Bosnia and Herzegovina, a number of imported animals infected by these bacterium species are registered during the after-war period. According to the reports of cantonal institutes for public health in Federation of Bosnia and Herzegovina, four brucellosis cases were registered during 2001, 14 during 2002, and there was a sudden increase to 47, during 2003. These parameters suggest that there are infected and ill animals on particular regions of Federation B&H, which disseminate causal agents in their near environment, which are transmitted to humans by contact, aerosols, animal excretions, milk, and milk products. In our review of the infected patient with brucellosis, we emphasized the importance of the disease, the way of treating, as well as the kinds of biology material from which it is possible to isolate the causal agent. We also indicated the importance of serol-

ogy methods that can detect the presence of specific antibodies in the patient's serum, and in this way confirm or reject a clinical suspicion of this disease. According to available data, it is clear that brucellosis and causal agent of the disease persist within susceptible animal population, which results in occasional episodes of infections or abortions of a number of animals. Human brucellosis cases are always narrowly related to bacterial persistence within populations of susceptible animals, since the bacteria live in their milk glands and reproductive organs, from which they are excreted in external environment. In our described case, there was also a narrow relation between working with animals and disease occurrence. In order to put brucellosis, as well as similar diseases, under a medical control, it is necessary to have professionally developed veterinarian service in particular areas, which would observe and take appropriate steps for preventing the outbreak of the disease, in cooperation with other services. It is also important to emphasize that the disease has a subacute-chronic progress, which can develop into the chronic progress in a small number of cases, which depends on a causal agent, as well as on appropriate kind and duration of antibiotic therapy.

CONCLUSION

- The work diagnosis of brucellosis is confirmed microbiologically and serologically.
- Bacterium *Brucella melitensis* is isolated and identified, as a final result.
- The presence of specific IgM and IgG antibodies is confirmed in the sera, quantitatively and qualitatively.
- The results of laboratory tests confirmed etiological causality of the disease with isolated bacterium.

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