OUTBREAK OF HUMAN BRUCELLOSIS IN BOSNIA AND HERZEGOVINA: EVALUATION AND IMPORTANCE OF MICROBIOLOGICAL METHODS FOR THE DIAGNOSIS OF BRUCELLOSIS

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ABSTRACT

Purpose: Brucellosis is a worldwide zoonosis. In Bosnia and Herzegovina first cases of brucellosis were registered in 2000, since than disease is in an expansion. After the implementation of the program for mass vaccination of animals in 2009, the incidence of human brucellosis rapidly decreased, but only a few years. After some period of decreasing brucellosis, in 2017 and 2018 a new outbreak of brucellosis was registered. The aim of this study was to analyze epidemiological characteristics of patients in the last outbreak of brucellosis and diagnostic value of blood culture, Rose Bengal test and immunoenzymatic test (ELISA IgM and IgG).

Patients and methods: This study included 181 brucellosis patients from January 2017 to September 2018. The disease was diagnosed by positive blood culture results, or by positive and relevant serologic test results.

Results: The average age of patients was 45.72 years of life-range from 1 to 78. The gender distribution of patients was: 123/181 (67.96%) males and 58/181 (32.04%) females. Of the total of 181 studied patients, blood culture was requested for 112 (61.8%) of patients and in 37/112 (33%) were positive. The Rose Bengal test was positive in all patients 181/181(100.0%). Brucella IgM antibodies with ELISA were positive in 64/181(35.35%), while IgG antibodies were positive in 161/181(88.95%) of patients.

Conclusion: Our results showed that sensitivity of test methods was different in the different stages of illness and so only combination of blood culture, the Rose Bengal test and ELISA ensured early and precise diagnosis of human brucellosis.

Keywords: brucellosis, infection, laboratory diagnosis.

INTRODUCTION

Brucellosis is a worldwide zoonosis with a high degree of morbidity in humans. The disease became endemic in many countries, especially in the Mediterranean region [1-5]. In Bosnia and Herzegovina first cases of brucellosis were registered in 2000, since than disease is in an expansion. There are several reasons why brucellosis and other zoonoses became a frequent occurrence in our country.

Some include the import of livestock from abroad without taking preventive measures, and even more important is the import of cattle across the unguarded borders. After the implementation of the program for mass vaccination of animals in 2009, the incidence of human brucellosis in our country rapidly decreased, but only a few years. Because most of the sheep had not been vaccinated, brucellosis quickly spread among the animals. Moreover, because the vaccination program has not been consistently carried out in BiH [5], during the past year, a significant number of new animals had been acquiring brucellosis and consequently pose a threat to humans. One of the causes of the persistent occurrence of animal and human brucellosis is poor veterinary and health care collaboration [6, 7]. Consequently, after some period of decreasing brucellosis, in 2017 and 2018 outbreak of brucellosis was registered in three cantons of the Federation of Bosnia and Herzegovina: Central Bosnia, Sarajevo and Zenica-Doboj.

Clinical findings in human brucellosis are non-specific, such as fever, headache, sweat, anorexia and back pain. Clinical polymorphism is very common and for this reason brucellosis is often unrecognized in primary health care settings [5]. Diagnosis of brucellosis is based on the clinical picture, epidemiological and anamnestic data and laboratory analysis.

The aim of the study was to analyze epidemiological characteristics of patients in the last outbreak of brucellosis in B&H and the importance of microbiological methods in the early and precise diagnostic of human brucellosis.

PATIENTS AND METHODS

The study was conducted between January 2017 to September 2018. The investigation was performed at the Department of Clinical Microbiology, Clinical Centre University of Sarajevo.

Patients

This study included 181 brucellosis patients from different regions of the Federation B&H. All patients were treated at the Clinic for Infectious Diseases, Clinical Centre University of Sarajevo. We analyzed gender, age, profession, and way of transmission of the disease among brucellosis patients.

Methods

The disease was diagnosed by positive blood culture results and/or by positive and relevant serologic test results. The study included only patients in which all three methods (blood culture, Rose Bengal test and immunoenzymatic test) were applied. Blood cultures were performed by inoculation of 8-10 ml of freshly collected blood into aerobic BACTEC bottle and incubation for up to 21 days in the "BACTEC 9120" semi-automated systems (Becton Dickinson Diagnostic Instruments Systems, Maryland, USA). Bottles giving a positive growth index were Gram stained and subcultured to blood agar plates. *Brucella* isolates were identified by conventional biochemical testing (catalase, oxidase and urease activity; glucose fermentation and production of H₂S). *Brucella* spp. suspected isolates were confirmed by slide agglutination using type-specific antisera (Murex Diagnostics, Dartford, United Kingdom).

For serology, blood samples were centrifuged and the serum divided into refrigerator and stored at -20°C until tested. All sera were evaluated using the Rose Bengal test and ELISA (IgM and IgG). The Rose Bengal plate agglutination (RB test) was performed according to manufacturer's instructions (bio Merieux, Marcy L'Etoile/ France). *Brucella* IgM and IgG enzyme-linked immunosorbent assays (ELISAs) were obtained from Genzyme Virotech GmbH/Germany. The test was performed and evaluated according to the kit procedure. The test result was read automatically by BEP 2000/Behring ELISA processor.

Statistical analysis

For evaluation of the results, standard statistical methods were used. Normality of the continuous variables was tested by the Kolmogorov-Smirnov test. The test showed that all variables satisfied the characteristics of normal distribution. Chi-square test was used to examine statistical significance for categorical variables. Correlation between the investigated variable was found using Pearson's coefficient linear correlation. Statistical significance was defined at $p < 0.05$. Statistical analysis were performed by SPSS software package (Version 19.0 for Windows).

Ethical principles

All procedures followed were in accordance with the ethical standards promoted in the 1964 Declaration of Helsinki and its later amendments.

RESULTS

In this study, we analyzed the last outbreak of human brucellosis in B&H. The study included 181 patients observed between January 2017 and September 2018. The average age of patients was 45.72 years of life-range from 1 to 78; the most of cases were between 41 and 50 years of age (Graph 1.). The gender distribution of patients was: 123/181 (67.96%) males and 58/181 (32.04%) females. The majority of patients 87/181 (47.80%) were directly involved in sheep farming (adult and farmers children) and had contact with sheep during lambing (Graph 2.). In addition, 142/181 (78.70%) patients consumed the tradition-

ally prepared sheep cheese and milk without prior thermal processing.

Graph 1. Review of the patient's age

Graph 2. Review of professional structure of brucellosis patients

We evaluated the diagnosis of brucellosis by blood culture, the Rose Bengal test and ELISA (IgM and IgG). Of the total of 181 studied patients, blood culture was requested for 112 (61.8%) of patients and in 37/112 (33%) were positive. The Rose Bengal test was positive in all patients 181/181 (100.0%). *Brucella* IgM antibodies with ELISA were positive in 64/181 (35.35%), while IgG antibodies were positive in 161/181 (88.95%) of patients (Graph 3.). We analyzed the correlation between diagnostic methods: our results showed that value of blood culture and IgM antibodies were in the correlation in 59.3% of the patients: χ^2 demonstrated statistical significant co-

incidence among blood culture and IgM ($p=0.001$). Results showed that the value of IgM and IgG were in the correlation in 20.9% of the patients. χ^2 demonstrated statistical significant distinction among the value of IgM in relation

to value of IgG ($p=0.001$). As we compared the results of the test methods according to the appearance of symptoms, we concluded that results were different at different stages of illness (Graph 4.).

Graph 3. Results in percentages of different diagnostic tests

Graph 4. Review of results different diagnostic tests at different stages of illness.

DISCUSSION

Brucellosis represents a prevalent disease in humans and animals in our country. In B&H first cases of brucellosis were registered in 2000, since than disease is in an expansion. Brucellosis shows a tendency to increase and become the most important public health problem [3, 5].

As the clinical picture in human brucellosis is fairly non-specific, a clinical diagnosis of the disease is difficult. Therefore, its exact diagnosis necessitates the use of specific diagnostic methods.

In this study, we analyzed the last outbreak of human brucellosis in B&H. The study included 181 patients. The average age of patients was 45.72 years of life-range from 1 to 78. Infections were reported in all age groups, but they occurred predominantly in the 41-50 years group. The gender distribution of patients was: 123/181 (67.96%) males and 58/181 (32.04%) females. The majority of human brucellosis cases occurred in men, as observed in other Balkan countries [3, 5, 10]. Analysing of the profession of brucellosis patients showed that 87/181 (47.80%) were di-

rectly involved in sheep farming (adult and farmers children) and had close contact with sheep during lambing. The principal modes of transmission were consumption of the traditionally prepared sheep cheese and milk without prior thermal processing and occupational contact with infected animals, as observed in a similar study [12].

We evaluated the effectiveness of different test methods: blood culture, the Rose Bengal test and immunoenzymatic test (ELISA IgM and IgG). Only one specimen for any type of test in each patient were examined because the purpose of the study was to analyze the effectiveness of test methods at different stages of illness. Of the total of 181 studied patients, blood culture was requested for 112 (61.8%) of patients and in 37/112 (33%) were positive. Positive blood cultures were detected very early, in 6% of patients in the first week of infection. Blood cultures were positive during the first three months of infection, 92% during the first month. This finding suggested that blood culture is the method of choice for definitive diagnosis in the acute phase of the disease. Microbiological isolation re-

mains the “gold standard” for the diagnosis of brucellosis and sensitivity of this method varies from 15 to 70% [13, 14, 15]. Blood culture demonstrated high sensitivity in patients with primary infections but this technique has the limitations as a laboratory test. Brucellosis is one of the most common laboratory-acquired infections because isolation of the bacteria is hazardous. This microbiological method needs especially expensive laboratory equipment. Therefore, in a rural poor area where brucellosis is endemic, diagnosis of brucellosis is based on different serological tests [16]. Different serological tests demonstrate different advantages or deficiency. The Rose Bengal test has a high degree of sensitivity for the diagnosis of brucellosis, irrespective of the stage of the disease. This is very simple and rapid plate agglutination test (4 min.). Therefore, the Rose Bengal test is ideal for screening patients for human brucellosis. In our study the Rose Bengal test was positive in all patients 181/181 (100.0%). These results were expected because in this study only brucellosis patients were included. Although the Rose Bengal test has a lot of advantages, it is great mistake to establish the treatment of brucellosis in endemic areas by using test as the sole diagnostic tool, especially with individuals who are exposed repeatedly to infection [17]. This test suffers from a high false-negative rate in complicated and chronic cases [17]. Because of the limitations of the Rose Bengal test, other assays, especially ELISA, which can determine the classes and subclasses of immunoglobulins in a sensitive and simple manner, can be used as confirmatory tests [13, 18, 19]. Brucella IgM antibodies with ELISA were positive in 64/181(35.35%), while IgG antibodies were positive in 161/181(88.95%) of patients. IgM antibodies were detected during the first week of infection. The peak level was detected 4 weeks after exposure. IgG antibodies were detected 25 days after exposure to infection, 2 weeks after IgM antibodies. The peak level for IgG antibodies was detected after 3 months. In a few 2% patients IgG antibodies persisted longer than 12 months. This fact suggested that among these patients, chronic form of brucellosis was developed. Several studies have shown that ELISA is the test of choice for the diagnosis of complicated and chronic cases, especially when other tests are negative [18, 19].

The data obtained from our study indicate that brucellosis was diagnosed in the different phase of the disease. Unfortunately, in the most cases, patients were ill a prolonged period before the diagnosis was established. In some of the patients brucellosis was diagnosed as late as 12

month after the first symptoms appeared. In cases where the doctor suspected brucellosis and used corresponding diagnostic methods, the diagnosis of brucellosis was made in an early stage of illness. Early and precise diagnosis and inclusion of adequate antibiotic therapy have crucial importance for patients, especially for protection development complications of brucellosis. Results of this study showed that brucellosis was detected in the early stage of illness in only 1/3 of patients. Although patients visited the primary health care unit, the disease was not recognized. Unfortunately, no tests for brucellosis were applied.

As we compared results of test methods according to symptoms appeared, we showed that results were different in the different stages of illness, the effectiveness of test methods was different in the different stages of illness. Therefore, only a combination of blood culture, the Rose Bengal test and ELISA (IgM, IgG) ensured early and precise diagnosis of human brucellosis.

It is evident that brucellosis shows a tendency to become the most important public health problem in B&H and so the national strategy for brucellosis control is necessary. Controlling brucellosis requires planning at the national level. Cooperation between the veterinary and health sectors must be better. Health education for health professionals, veterinarian professionals, and the general population is necessary. Adequate prevention of brucellosis requires a multi-disciplinary approach with close cooperation between veterinarians, microbiologists, infectious disease specialists, epidemiologists and public health professionals [5]. Experience of other countries where an organized and financially supported strategy was accomplished may be good guidelines for the management of human brucellosis [20, 21].

CONCLUSION

In Bosnia and Herzegovina brucellosis shows a tendency to increase and become the most important public health problem. Diagnosis of brucellosis is based on the clinical picture, epidemiological data and laboratory analysis. The present study deals with usefulness and significance of specific microbiological tests. The data obtained from our study indicated that the effectiveness of test methods are different at different stages of illness. Therefore, only combination of blood culture, the Rose Bengal test and ELISA (IgM, IgG) ensured early and precise diagnosis of human brucellosis.

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