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Original Article

Epidemiological, clinical, biochemical, and treatment characteristics of brucellosis cases in Turkey

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Abstract

Introduction: In our study, we aimed to evaluate the epidemiological features of brucellosis and the efficacy of different treatment options in patients with various organ involvements.

Methodology: Patients diagnosed with brucellosis and treated in two different centers between 2009 and 2019 were retrospectively screened and evaluated regarding epidemiological and clinical features, laboratory findings, and treatment responses.

Results: The study included 297 complete-data patients (76% of rural patients were farmers). Farming (76%) and raw dairy (69%) were the main transmission methods. Most patients (98.6%) had positive tube agglutination tests. Ninety-two patients' blood and bodily fluid cultures grew Brucella spp. The incidence of leukopenia was 18.8%, thrombocytopenia 10.7%, anemia 34.3%, and pancytopenia 4.3%. Doxycycline and rifampicin were the major treatments, with streptomycin utilized in osteoarticular patients. Pregnant women with neurobrucellosis took ceftriaxone and trimethoprim-sulfamethoxazole. After one year, 7.1% of patients relapsed. Doxycycline + streptomycin and doxycycline + rifampicin had similar relapse rates ($p = 0.799$). The double- and triple-antibiotic groups had identical recurrence rates ($p = 0.252$).

Conclusions: In uncomplicated brucellosis cases doxycycline + streptomycin and doxycycline + rifampicin treatments were equally effective. Again, there is no statistical difference in relapse development rates between double and triple combination treatments in uncomplicated brucellosis cases. Relapsed patients generally miss follow-ups, interrupt therapy, have osteoarticular involvement, and get short-term treatment. Patients with focused participation should be thoroughly checked at diagnosis and medicine, and treatment should be lengthy to prevent relapses.

Key words: Brucellosis; epidemiology; treatment; relapse; anti-bacterial agents.

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Introduction

Brucellosis is a systemic zoonotic infection with bacteria of the *Brucella* spp genus and can affect many organs and systems. It is often transmitted to humans by infected products of animals such as sheep, cows, goats, and pigs, by consumption of unpasteurized milk or dairy products, direct contact with infected animals, or by inhalation [1]. Although brucellosis is seen worldwide, it is endemic, especially in developing countries. It is also an occupational disease for livestock practices, slaughterhouses, and laboratory workers. The condition is common in Mediterranean countries, the Arabian Peninsula, India, Mexico, and Central and South America. While brucellosis seropositivity is between 2% and 8% in the healthy population, it can increase to 25% in risk groups [2]. Although 500,000 new cases of brucellosis are reported annually worldwide, it is estimated that 26 times this number are at risk [3]. Although mortality rates are low, morbidity is quite high. Therefore, a public health problem can cause significant economic losses, especially in developing countries [4].

In this disease, which may progress with multi-systemic involvement, the most common symptoms are

fever, arthralgia, malaise, sweating, headache, muscle pain, loss of appetite, low back pain and back pain, weight loss, and nausea-vomiting. Rifampicin, doxycycline, trimethoprim-sulfamethoxazole, ciprofloxacin, streptomycin, gentamicin, and ceftriaxone are effective agents in the treatment, and the application of specific combinations of these agents instead of monotherapy increases the success of the treatment and prevent relapse [5].

In our study, we aimed to evaluate the epidemiological features of brucellosis, as well as evaluate the efficacy of different treatment options in patients with various organ involvements.

Methodology

The data of brucellosis patients between 2009 and 2019 in Sabuncuoğlu Şerefeddin Training and Research Hospital of Amasya University and Farabi Hospital Infectious Diseases and Clinical Microbiology Clinics of the Faculty of Medicine of Karadeniz Technical University were retrospectively analyzed. The study included individuals who were 18 or older and exhibited *Brucella* spp growth in blood culture and/or had titers of 1/160 and above in the standard tube agglutination (STA) test for *Brucella*. The following information was recorded on the study forms: Demographic characteristics of the cases, complaints at the time of admission, system inquiries, physical examination findings, complete blood count, C-Reactive Protein (CRP) levels, STA, blood and bone marrow cultures, liver and kidney function tests, radiological imaging results, treatment combinations applied and their durations. Brucellosis was diagnosed in clinically compatible patients with an STA result of ≥ 1/160 and/or blood, bone marrow, cerebrospinal fluid (CSF), and sperm culture. In addition, glucose, protein, cell count, Wright's stain, and culture tests were performed in the CSF sample taken by a lumbar puncture for neurobrucellosis in patients with central nervous system involvement symptoms such as headache, personality change, and confusion. Contrast-enhanced magnetic resonance imaging was requested from the patients in the presence of lumbar-hip joint pain showing osteoarticular involvement and symptoms such as swelling, pain, redness, and limitation of movement in any joint. The diagnosis of epididymo-orchitis was confirmed by scrotal ultrasonography in patients with scrotal pain and swelling, and it was accepted as genitourinary system involvement. Anemia was defined as a hemoglobin value of < 12 g/dL in females and < 13 g/dL in male patients. A leukocyte count < 4000/mm^3 was considered leukopenia and a leukocyte count > 10.000/mm^3 was considered as leukocytosis. A thrombocyte count < 150.000/mm^3 was considered thrombocytopenia, and cases with hematological disorders were considered as hematological involvement. Patients with increased serum transaminases more than 1.5 times the upper average value (when this increase could not be attributed to any other cause) were considered *Brucella* hepatitis.

The 1-year follow-ups of the patients after the end of the treatment were also examined. Patients who developed clinically relapsed brucellosis symptoms among these patients were considered relapsed if no other infectious focus was found.

Different combination regimens were applied to our patients, and these were doxycycline + rifampicin, doxycycline + streptomycin, doxycycline + rifampicin + streptomycin, rifampicin + ceftriaxone/co-trimoxazole and doxycycline + rifampicin + ceftriaxone/co-trimoxazole combinations.

Ethical consideration

The study was approved by the Amasya University Non-Invasive Clinical Research Ethics Committee. This study was conducted in a way to ensure the protection of patients (Document Date and Number: 26.05.2021-16782).

Statistical Analysis

SPSS version 25.0 (SPSS Inc. Chicago, IL, USA) was used for statistical analysis. The descriptive statistical analysis was performed for all the information obtained in the study. The Chi-square test was used in the analysis of categorical variables. Data obtained by measurement were expressed as mean ± standard deviation. The data obtained by counting were expressed as percentages (%). The conformity of the variables to the normal distribution was evaluated with the Kolmogorov-Smirnov test. The statistical analysis of normally distributed data was performed using the Student's t-test, and the statistical analysis of non-normally distributed data was performed using the Mann-Whitney U test. A p value < 0.05 was considered statistically significant.

Results

Of 297 patients, 196 (66%) were male, 101 (34%) were female, and the mean age was 43.8 years (age range: 18-85 years). Two hundred twenty-four (76%) patients lived in rural areas and made a living from animal husbandry. Two patients were veterinarians, and

one patient was a laboratory worker. The most common mode of transmission was animal husbandry and the use of unpasteurized milk-dairy products, 224 (76%) and 204 (69%) patients, respectively (Table 1). It was observed that 193 (65%) cases applied in the six months between March and August. One hundred sixty-six (56%) patients presented with acute, 71 (24%) with subacute, and 60 (20%) with chronic brucellosis clinical picture.

The most common symptoms detected in our cases at admission were fever, malaise, sweating, myalgia, arthralgia, and anorexia. The most common physical examination findings were fever, hepatomegaly, splenomegaly, scrotal pain, and swelling (Table 2). STA test was detected as ≥ 1/160 in 293 (98.6%) patients. Culture tests were performed in 204 patients, and *Brucella* spp. was grown in blood and other body fluids (bone marrow, CSF, and sperm) cultures in 91 (30.6%). The STA test of four patients was < 1/160, and the growth made the diagnosis of these patients of the bacteria in blood or bone marrow cultures.

Two hundred nineteen (73.7%) cases had elevated CRP, of which 106 (35%) had a CRP value less than five times the upper limit (0-0.5 mg/L), and 63 (21%) higher than 5-10 times the upper limit of CRP, and 50 (17%) had a CRP value elevated more than ten times the upper limit. In addition, the erythrocyte sedimentation rate (ESR) was high in 175 (58.9%) patients. Table 2 shows the clinical and laboratory data of the patients.

Hematologic involvement was seen the most in our patients. At least one hematological finding was detected in 154 patients (52%), leukocytosis was detected in 25 (8.5%), leukopenia in 56 (18.8%) patients, anemia in 102 (34.3%) patients, thrombocytopenia in 32 (10.7%) patients, and pancytopenia in 13 (4.3%) patients. Osteoarticular involvement (sacroiliitis, discitis, spondylodiscitis, paraspinal abscess) ranked second. Sixty-six (22.2%) patients had at least one of these osteoarticular involvements. Sixty-two (20.8%) patients had gastrointestinal system complaints.

Unexplained transaminase elevation (Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT)) was detected in 122 (41.1%) patients, and it was considered to be *Brucella* hepatitis.

In 21 (7.1%) patients, the presenting complaint was scrotal pain and swelling, and the diagnosis of epididymo-orchitis was confirmed by USG (ultrasonography), and STA was positive at a titer of ≥ 160 in these patients. In addition, *Brucella* spp. was grown in the sperm culture of one patient.

Eight (2.6%) patients presented signs of central nervous system involvement. Lymphocyte dominance in the CSF examination supported the diagnosis of

Table 1. Demographic and epidemiological characteristics of brucellosis cases.

Characteristics	n (297)	(%)
Age	43.8 (18-85)	
Gender		
Female	101	34
Male	196	66
Possible route of transmission		
Livestock and rural life	224	76
Use of unpasteurized milk and dairy products	204	69
Undetectable	72	24
Veterinary surgeon	2	0.6
Infected in laboratory	1	0.3
Season		
Spring (March, April, May)	99	33
Summer (June, July, August)	94	31
Autumn (September, October, November)	58	19
Winter (December, January, February)	46	17

Table 2. Common application symptoms, physical examination, and laboratory findings in brucellosis cases.

Characteristics	n (297)	%
Symptoms		
Malaise and fatigue	281	94.6
Fever	257	86.5
Joint pain	241	81.1
Anorexia	235	79.1
Sweating	223	75.1
Low back pain	146	49.1
Muscle pain	140	47.1
Headache	92	30.9
Hip pain	90	30.3
Stomach ache	62	20.8
Scrotal swelling and pain	21	7.1
Physical examination findings		
Fever	196	65.9
Weight loss	161	54.2
Hepatomegaly	58	19.5
Splenomegaly	31	10.4
Hepatosplenomegaly	28	9.4
Sacroiliitis	24	8.1
Epididymo-orchitis	21	7.1
Meningeal irritation	8	2.6
Laboratory findings		
Anemia	102	34.3
Leukocytosis (> 10 000/mm^3)	25	8.5
Leukopenia (< 4000/mm^3)	56	18.8
Thrombocytopenia (< 150 000/mm^3)	32	10.7
Pancytopenia	13	4.3
Transaminase elevation	122	41.1
CRP elevation	219	73.7
ESR elevation	175	58.9
STA positivity	293	98.6
Culture positivity	91	30.6

CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; STA: Standard tube agglutination.

neurobrucellosis in addition to the clinical findings and epidemiological features of these patients. In all of our patients diagnosed with neurobrucellosis, STA was found to be ≥ 1/160.

All patients were given the standard treatments recommended in the guidelines, and 21 (7.1%) patients had repeated complaints of brucellosis, such as fever, malaise, and joint pain within the first year. These symptoms and complaints could not be attributed to any other cause and were defined as a relapsed brucellosis case. The most preferred combination in the treatment of patients was doxycycline 2 × 100 mg + rifampicin 1 × 600 mg in 206 (69.4%) cases. The cure was achieved in 190 (92.2%) of these patients, and relapse was observed in 16 (7.8%) patients. Doxycycline 2 × 100 mg + streptomycin 1 × 1 g was used in 10 patients, and one patient relapsed.

The combination of doxycycline 100 mg q12H + rifampicin 600 mg once daily + streptomycin 1 g once daily was administered to 66 patients with osteoarticular involvement. In three patients with hearing loss, streptomycin was discontinued in a shorter time, while it was given for 21 days in other patients. No relapse was observed in patients whose streptomycin treatment was terminated early. Doxycycline + rifampicin treatment was continued until clinical and radiological improvement was achieved. Although 63 (95.5%) of 66 patients were cured, 3 (4.5%) had relapses.

A combination of rifampicin and ceftriaxone/co-trimoxazole was used in seven pregnant patients. Six of these seven pregnant patients were treated, and relapse was observed in one.

The doxycycline + rifampicin + ceftriaxone combination was used for one month in eight patients diagnosed with neurobrucellosis. Treatment was continued with rifampicin and doxycycline until the clinical findings of the patients improved and CSF findings returned to normal. No relapse was observed in any of these patients. The treatment regimens applied to the patients and the relapse rates are given in Table 3.

It has been determined that relapse cases are chronic cases that do not attend their follow-ups regularly, disrupt their treatment, and present with osteoarticular involvement (especially spondylodiscitis and vertebral osteomyelitis). In relapsed cases, treatment was generally provided with a combination of streptomycin 1 g once daily for 21 days + rifampicin 600 mg once daily + doxycycline 100 mg q12H for three to six months. There was no statistical difference between the patient's demographic, clinical, and laboratory findings with and without relapse ($p > 0.05$). The relapsed patients received 4.8 ± 2.4 months of treatment, non-relapsed patients received 3.2 ± 2.5 months, and the relapsed patients received statistically significantly longer treatment ($p = 0.001$). There was no statistical difference in relapse development in patients using the doxycycline + rifampicin combination and patients using doxycycline + streptomycin ($p = 0.799$). There was no statistical difference in relapse development between patients who received dual antibiotic combinations and triple combination (doxycycline + rifampicin + streptomycin) ($p = 0.252$).

Discussion

Brucellosis is one of the most common zoonoses in the world. Although the disease affects all age groups and genders, it is more common in regions where animal husbandry is common [1,6]. Human brucellosis is common worldwide, with more than 500,000 new cases per year, except for northern, western, and central Europe, some Asian and American countries, Australia, and New Zealand [7-10]. Brucellosis is endemic in Syria, Iraq, Saudi Arabia, Turkey, and Iran, which have the highest incidence rates in the world and most of the Middle Eastern countries [11]. The incidence of Brucellosis in Turkey varies geographically between 1% and 26.7% [2]. It is estimated that the actual incidence is higher due to the variability of the brucellosis clinic and its ability to imitate many diseases.

Data based on statistics or information passively collected from hospitals and diagnostic laboratories are often incomplete. The incidence is estimated to be 26 times higher than reported due to misdiagnosis and underreporting [3,10]. *Brucella* is transmitted from animals by direct contact or by consumption of infected milk and cheese [12]. Our study determined that most cases live in rural areas, deal with animal husbandry, and consume unpasteurized milk and dairy products. In our country, 50-60% of the cases are between 20 and 50

Table 3. Treatment schemes and relapse rates in brucellosis cases.

Treatment regimens	Diagnosis of brucellosis	n (%)	Relapse n (%)
Doxycycline + rifampicin	Uncomplicated brucellosis	206 (69.4)	16 (7.8)
Doxycycline + streptomycin	Uncomplicated brucellosis	10 (3.3)	1 (10)
Doxycycline + rifampicin + streptomycin	Osteoarticular brucellosis	66 (22.2)	3 (4.5)
Rifampicin + ceftriaxone/ co-trimoxazole	Pregnancy + brucellosis	7 (2.4)	1 (14.3)
Doxycycline + rifampicin + ceftriaxone	Nörobrucellosis	8 (2.6)	0 (0)

years old, 10-15% are in children, and 10% are over 65 years old [13]. In our study, the mean age of the patients was 43.8 years. The reason for clustering the cases in the young-adult period is that the people dealing with agriculture and animal husbandry are usually at this age. Although veterinarians, farmers, shepherds, the meat and dairy industry, and laboratory workers are in the risk group for brucellosis, the most common transmission is through food sources [14]. In our study, the most common transmission routes were animal husbandry, living in rural areas (76%), and unpasteurized milk and dairy products (69%). Two patients are veterinarians, and one is a member of the occupational group at risk for transmission as a laboratory worker.

Although the disease can be seen all year round, it is more common in spring and summer due to people traveling to rural areas, the calving period of sheep and cattle, and the increase in fresh cheese production and consumption [15]. For example, a study conducted in Iran reported that the incidence in spring, summer, autumn, and winter was 38%, 29%, 18%, and 15%, respectively [16]. In our study, in parallel with these data, the seasonal incidences of *Brucella* cases were determined as 33%, 31%, 19%, and 17%, respectively.

Symptoms and clinical findings in brucellosis are not specific to the disease. For this reason, brucellosis may be confused with many conditions. While the cases may present with a noisy clinical picture with acute fever, they may also present with a slower and chronic course of infection such as malaise, loss of appetite, weight loss, and chronic low back pain and hip joint pain. While the most common symptoms detected during admission are arthralgia, fever, and malaise, the most common physical examination findings are fever and hepato/splenomegaly [4,17]. It has been reported in studies that the symptoms are seen at very different rates: fever (55-100%), malaise (33-97%), sweating (19-96%), arthralgia (17-87%), hepatomegaly (6-55%) and splenomegaly (7-69%) [18]. Our study observed that 94.6% of the cases had malaise and fatigue, 86.5% had a fever, 81.1% had joint pain, and 79.1% had anorexia. Among the physical examination findings, hepatomegaly, splenomegaly, and hepatosplenomegaly were found at 19.5%, 10.4%, and 9.4%, respectively. Twenty-one (7.1%) patients had signs of epididymo-orchitis at admission, and almost all of these patients made their first application to urology clinics. In a recently published meta-analysis, the existence of asymptomatic brucellosis cases is mentioned [19]. There was no asymptomatic case in our study.

Routine laboratory results are generally not diagnostic in brucellosis. Leukopenia, anemia, and thrombocytopenia may be observed due to the infection affecting the bone marrow. Elevated transaminases may be regarded as a result of the disease affecting the liver, and these results may be expected. Elevated acute phase reactants such as CRP and ESR are among the most frequently reported laboratory abnormalities [4,20]. Different rates of hematological involvement have been reported in studies. A study in our country noted that anemia was 20.4%, thrombocytopenia was 15.5%, leukopenia was 12.1%, CRP elevation was 63.1%, and ESR elevation was 58.7% [21]. A study by Zheng *et al*. showed that anemia developed in 23.9% of the cases, leukopenia in 24.1%, thrombocytopenia in 15.8%, and pancytopenia in 13.2% [22]. In our study, anemia was found to be 34.3%, leukopenia 18.8%, thrombocytopenia 10.7%, and pancytopenia 4.3%. Leukocytosis was detected in 25 (8.5%) patients. At the time of diagnosis, 73.7% of our cases had elevated CRP, and 58.9% had elevated ESR. These high values were observed to decrease to normal levels thanks to the treatment, and they were used together with clinical findings in evaluating treatment response. A study by Şahintürk *et al.* stated that 36% of 195 patients diagnosed with Brucellosis had AST or ALT elevations before starting the treatment [23]. In a case series reported from Italy, it was reported that transaminase elevation was detected in 17% of the cases [24]. In our study, other causes of transaminase elevation were investigated in cases where transaminase elevation was seen at the time of diagnosis. Patients whose transaminase elevation could not be explained by another reason were considered to have *Brucella* hepatitis. Transaminase elevation in our cases was found to be 41.1%. The diagnosis is made by clinical findings, serology, and definitive isolation of the microorganism (blood, bone marrow, cerebrospinal fluid, sperm, tissue culture). In the absence of bacteriological confirmation, serological tests often make the diagnosis. Demonstration of the presence of specific antibodies, as well as an increased titer of these antibodies, is diagnostic. The most commonly used serological test is the STA test [25,26]. In a study with 184 patients in which serological and bacteriological methods confirmed Brucellosis, the STA positivity rate was 83.7% [27]. Another study found the *Brucella* standard tube agglutination test 100% specific and sensitive at a titer of ≥ 1/160 [28]. However, it should not be forgotten that the STA titer may be low in the presence of relevant clinical findings. It is emphasized that in cases where STA titers are < 1/160, it would be

wrong to evaluate the STA result as unfavorable without following the patient's clinical signs and titer increase [29]. In our study, although the STA titers of two patients admitted in the acute period were 1/40 and 1/80, the diagnosis was made by growing the agent in blood cultures. It was observed that these titers increased to 1/160 and 1/320 in both patients in the follow-ups. In our study, the STA test was positive in 293 (98.6%) cases. As stated in the study of Alsubaie *et al*., there is no correlation between agglutination test titers and culture positivity [30]. In our study, brucellosis was diagnosed with blood or bone marrow culture positivity in four cases with STA test < 1/160. In 92 of our patients, the agent was produced in blood and bone marrow cultures, and in one of our cases with epididymo-orchitis, the agent was isolated in the ejaculate culture. The culture positivity rate in our cases was 31%.

Tetracycline, doxycycline, minocycline, aminoglycosides (amikacin, gentamicin, and streptomycin), quinolones (ciprofloxacin), rifampin, ceftriaxone, and trimethoprim/sulfamethoxazole are effective drugs against *Brucella* spp [31]. Despite extensive studies, the optimum antibiotic treatment for brucellosis is still controversial. For uncomplicated brucellosis, the World Health Organization (WHO) recommends a combination of doxycycline 200 mg daily for at least six weeks and streptomycin 1 g daily for 2 to 3 weeks to treat adult acute brucellosis. The recommended alternative treatment is doxycycline 200 mg/day for six weeks and rifampicin 600-900 mg/day [32] which was shown effective in our study with a relapse rate of only 7.8%. Of note, a similar previous also showed that this regimen is effective for uncomplicated brucellosis and comparable to the regimen involving aminoglycosides [33]. However, a recent systematic review with meta-analysis found that triple therapy is more effective [34]. In our study, the duration of the treatment was six weeks in patients without focal involvement and three to six months in patients with focal involvement (particularly spinal region involvement). It has been reported in different studies that relapses may develop at rates ranging from 3.3% to 11.6% after the treatment [4-6]. Treatment failure and relapse are not usually due to antibiotic resistance. Conditions predisposing to relapse are inadequate treatment, ineffective antibiotic therapy, positive blood culture at the onset of the disease, male gender, and a platelet count of less than 15000/mm^3 [19]. In addition, not informing the patients about treatment adherence and not following the patients closely were stated as factors related to relapse [4]. The relapse rates in our cases were found to be 7.1%. Our patients with relapse generally lived in rural areas, had limited access to quality healthcare services, had a low socioeconomic level, and did not attend regular check-ups. Notably, 13 (62%) of 21 patients with relapse developed complications in the spinal region, such as spondylitis, spondylodiscitis, and epidural abscess at the first admission. Therefore, patients diagnosed with brucellosis should be carefully examined regarding spinal region involvement at the time of diagnosis and during treatment. Patients with spinal region involvement should be followed closely after the treatment against the development of relapse. In these patients, the duration of the treatment should be extended, in addition to ensuring compliance with the treatment.

Limitations

A primary constraint of our study was the inability to examine blood culture in all participants. One additional limitation pertained to the challenge of assessing the treatment status of certain patients who did not consistently attend their scheduled follow-up appointments.

Conclusions

As a result, brucellosis continues to be a severe infectious disease worldwide. Much of the long and combined treatment required is done outside the hospital. For this reason, choosing drugs that are easy to obtain and administer will provide an advantage in patient compliance and treatment success. Although the WHO recommends the combination of doxycycline + streptomycin as the first choice in treating uncomplicated brucellosis cases, this combination is not superior to rifampicin + doxycycline and other triple combinations in post-treatment relapse development. The most essential factor in preventing the development of relapse is the patient's compliance with the treatment. In addition, we believe that continuing the treatment for three to six months by revealing the involvement of the spinal region at the time of diagnosis or during the treatment process will be beneficial in preventing relapses.

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