original article

Wien Med Wochenschr (2019) 169:232–239
https://doi.org/10.1007/s10354-019-0685-z

wmw
Wiener Medizinische Wochenschrift

Epidemiologic, clinical, and laboratory characteristics of childhood brucellosis

A study in an Iranian children's referral hospital

Babak Pourakbari · Mohamadreza Abdolsalehi · Shima Mahmoudi · Maryam Banar · Farbod Masoumpour · Setareh Mamishi

Received: 25 August 2018 / Accepted: 16 January 2019 / Published online: 18 February 2019

Summary
Background Brucellosis is endemic in Iran. Children constitute 20–25% of cases. We determined clinical, laboratory, and epidemiologic characteristics of pediatric brucellosis patients hospitalized at the Children's Medical Center from May 2011 to December 2016.
Methods Medical records were reviewed retrospectively. For each patient, a questionnaire was provided containing demographic characteristics (sex, age, nationality, date of admission, city of residence, history of ingestion of unpasteurized dairy products, family history of brucellosis, history of contact with suspicious animals) and clinical information (signs and symptoms, laboratory findings, history of disease relapse, treatment).
Results Included were 43 patients diagnosed with brucellosis (26 males, 60.5%; age 1–13 years, mean ± SD: 7.02 ± 3.5). A history of ingestion of raw or unpasteurized dairy products was present in 88% (*N* = 38) and 11 patients (26%) had had contact with a suspicious animal. Highest frequencies of brucellosis were recorded in 2013 (*N* = 10, 23%) and 2015 (*N* = 11, 26%). Most cases were admitted in the summer (*N* = 14, 33%) and spring (*N* = 12, 28%). Fever (*N* = 39, 91%), arthralgia (*N* = 33, 77%), and malaise (*N* = 33, 77%) were the main complaints. Anemia (65%), lymphocytosis (51%), and elevated erythrocyte sedimentation rate (86%) and C-reactive protein (67%) were the most prominent blood anomalies. Blood culture was positive in 30% (*N* = 11/37), bone marrow culture in 31% (*N* = 4/11). A positive Wright, Coombs Wright, and 2 ME test was observed in 67% (*N* = 29), 92% (*N* = 34/37), and 85% (*N* = 34/40) of cases, respectively. Median length of antibiotic therapy was 12 weeks (2–24 weeks). The most frequent drug regimen was combined trimethoprim-sulfamethoxazole and rifampicin (*N* = 24, 56%). Relapse occurred in 9 patients (21%), there were no deaths.
Conclusions Physicians should be aware of the manifestations, diagnosis, and treatment protocols of childhood brucellosis. Control programs and preventive measures, e.g., regular examination of domestic animals, mass vaccination of livestock, slaughter of infected animals, control of animal trade and migration, pasteurization of milk and milk products, training and increased public awareness of the dangers of consumption of unpasteurized dairy products, are highly recommended.

Keywords *Brucella* · Children · Epidemiology · Zoonotic diseases · Disease transmission, infectious

B. Pourakbari · S. Mahmoudi · M. Banar · S. Mamishi
Pediatric Infectious Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran

M. Abdolsalehi · F. Masoumpour · S. Mamishi (✉)
Department of Infectious Diseases, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, No.62, Gharib St., Keshavarz Blvd., Tehran, Iran
smamishi@sina.tums.ac.ir

Epidemiologische, klinische und Laborcharakteristika der kindlichen Brucellose
Eine Studie in einem iranischen Schwerpunkt-Kinderkrankenhaus

Zusammenfassung
Grundlagen Die Brucellose ist im Iran endemisch. Kinder machen 20–25 % der Fälle aus. Die Autoren untersuchten von Mai 2011 bis Dezember 2016 klinische, laborbezogene und epidemiologische Merkmale

von kindlichen Brucellosepatienten am Children's Medical Center.
Methodik Krankenunterlagen wurden retrospektiv ausgewertet. Für jeden Patienten gab es einen Fragebogen mit demografischen (Geschlecht, Alter, Nationalität, Aufnahmedatum, Wohnort, Anamnese zum Verzehr unpasteurisierter Milchprodukte, Brucellose-Familienanamnese, Anamnese zum Kontakt mit krankheitsverdächtigen Tieren) und klinischen Informationen (Symptome, Laborergebnisse, Anamnese zu Krankheitsrezidiven, Therapie).
Ergebnisse Ausgewertet wurden die Daten von 43 Patienten mit der Diagnose einer Brucellose (26 m., 60,5 %; Alter: 1–13 Jahre, Mittelwert ± Standardabweichung: 7,02 ± 3,5). Anamnestische Hinweise auf den Verzehr roher oder unpasteurisierter Milchprodukte lagen bei 88 % (n = 38) vor, 11 Patienten (26 %) hatten Kontakt zu einem Tier mit Krankheitsverdacht gehabt. Die größten Häufigkeiten der Brucellose wurden 2013 (n = 10, 23 %) und 2015 (n = 11, 26 %) dokumentiert. Die meisten Fälle wurden im Sommer (n = 14, 33 %) und im Frühling (n = 12, 28 %) aufgenommen. Fieber (n = 39, 91 %), Arthralgie (n = 33, 77 %) und Unwohlsein (n = 33, 77 %) waren die Hauptbeschwerden. Anämie (65 %), Lymphozytose (51 %) sowie erhöhte Werte bei Blutsenkungsgeschwindigkeit (86 %) und C-reaktivem Protein (67 %) waren die auffälligsten Blutveränderungen. Die Blutkultur war in 30 % der Fälle positiv (n = 11/37), die Knochenmarkkultur in 31 % (n = 4/11). Ein positiver Wright-, Coombs-Wright- und 2-ME(Mercaptoethanol)-Test wurde in 67 % (n = 29), 92 % (n = 34/37) bzw. 85 % (n = 34/40) der Fälle beobachtet. Die mittlere Dauer der Antibiotikatherapie betrug 12 Wochen (2–24 Wochen). Das meistverwendete Therapieschema bestand aus der Kombination Trimethoprim-Sulfamethoxazol und Rifampicin (n = 24, 56 %). Ein Reziv trat bei 9 Patienten (21 %) auf, es gab keine Todesfälle.
Schlussfolgerungen Ärzte sollten sich der Symptome, Diagnose und Therapieschemata für Brucellose bei Kindern bewusst sein. Dringend empfohlen werden Regulierungsprogramme und Präventionsmaßnahmen, z. B. die regelmäßige Untersuchung von Haustieren, Massenimpfung des Viehbestands, das Schlachten infizierter Tiere, die Aufsicht über Tierhandel und -migration, Pasteurisierung von Milch und Milchprodukten, die Unterweisung und Erhöhung des öffentlichen Bewusstseins in Bezug auf die Gefahren des Verzehrs unpasteurisierter Milchprodukte.

Schlüsselwörter *Brucella* · Kinder · Epidemiologie · Zoonosen · Übertragung von Infektionskrankheiten

Brucellosis, also known as "undulant fever," "Malta fever," "Mediterranean fever," or "Gibraltar fever," is a widespread global zoonotic disease [1–3], which is endemic in Latin America, Southern Europe, Africa, Mediterranean basins, and the Middle East. It also has a high incidence rate among central Asian countries such as Kyrgyzstan and Azerbaijan [1, 3–5]. A recent systematic review conducted in Iran showed that the annual incidence rate of brucellosis in the country is still high (0.001%, 95% confidence interval [CI] = 0.0005–0.0015%) [6], and up to 25% of infected cases are children [1, 7].

The disease is caused by *Brucella* species, which are small, pleomorphic, fastidious, non-motile, gram-negative coccobacilli [1, 4, 8–12]. It is the primary infection of domestic and wild animals, including goats, sheep, camels, cows, pigs, and dogs, that can be transmitted to humans [4, 11, 13, 14]. Transmission occurs via ingestion of infected meat, raw unpasteurized milk, and dairy products; or through direct contact with infected animals and their secretions; or through the breathing of infectious aerosols [4, 5, 10–12, 14, 15]. Mother-to-child transmission is an infrequent but probable route of disease transmission [9].

Brucellosis involves multiple systems of the body and has various clinical manifestations such as fever, fatigue, arthralgia, muscle pain, rash, respiratory and cardiac complications, and orchitis/epididymitis [2, 11, 16]. The differences in manifestations among children and adults are not considerable. The disease affects children of any age group or gender [3], with its acute and sub-acute forms being more common in children [3, 12].

The purpose of this study was to determine the main clinical, laboratory, and epidemiologic characteristics of pediatric patients with brucellosis who were hospitalized at the Children's Medical Center (CMC) Hospital, a referral educational hospital in Tehran, Iran, during a 6-year period.

Patients and methods

This study was conducted at the CMC Hospital, Tehran, Iran, between May 2011 and December 2016. The medical records of all children with a definitive diagnosis of brucellosis admitted to the CMC Hospital were reviewed retrospectively. For each patient, a questionnaire was provided that contained demographic characteristics such as sex, age, nationality, date of admission, city of residence, history of ingestion of unpasteurized dairy products, history of brucellosis in the family, and history of exposure to infected animals, as well as clinical information such as signs and symptoms of the patient, laboratory findings, history of disease relapse, and applied treatment regimens.

The disease was diagnosed based on the presence of clinical signs and symptoms suggestive of brucellosis (such as fever, weight loss, anorexia, malaise and fatigue, arthralgia, sweating, nausea and vomiting, cough, etc.), together with positive serum agglutination tests (titers 1:160 or greater in the Wright test and

titers 1:40 or greater in Coombs Wright and 2-mercaptoethanol [2 ME] tests) or isolation of *Brucella* spp. from patients' blood or bone marrow cultures. Blood cultures were performed using the BACTEC 9120 Blood Culture System (Becton Dickinson & 48 Co., Franklin Lakes, NJ, USA). If no growth was seen after 7 days, incubation continued for 21 days, whereafter subcultures were inoculated on chocolate agar plates. Identification of *Brucella* spp. isolates was performed by Gram staining and conventional biochemical tests such as oxidase, catalase, urease production, methyl red/Voges–Proskauer (MR-VP) test, citrate utilization, indole test, motility (at both 37 °C and 20 °C), hydrogen sulfide production, etc. [17].

Laboratory workups such as complete blood cell (CBC) and differential counts, hemoglobin levels, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and liver function tests were performed for all patients and interpreted based on the reference values specific for infancy and childhood [18].

Neurobrucellosis was diagnosed according to the following principles: the presence of clinical symptoms (such as insomnia, amenorrhea, incontinence, neck stiffness, confusion, depression, etc.) in combination with the isolation of *Brucella* spp. from cerebrospinal fluid (CSF); or detection of anti-*Brucella* antibodies in the CSF; or detection of lymphocytosis, elevated levels of protein, and diminished levels of glucose in the CSF; and outcomes of cranial magnetic resonance imaging (MRI) or computed tomography (CT) scan [19].

A brucellosis relapse was considered as the re-emergence of brucellosis clinical manifestations together with the positive laboratory results (blood culture or serological tests) 2 months to 1 year after finishing the treatment [20].

Descriptive statistics were used to characterize patients' features. *P* values were determined using the chi-square test. A P value ≤ 0.05 was considered as significant. Analyses were performed using SPSS (version 16; SPSS Inc., Chicago, IL, USA) software.

Results

Of the total 8018 patients admitted to the infectious diseases ward of the CMC Hospital between May 2011 and December 2016, 43 patients were diagnosed as *Brucella*-infected cases (0.54%). Among them, 26 (60.5%) patients were male and 17 (39.5%) were female (Table 1). Patients' age ranged from 1 to 13 years (mean ± standard deviation [SD]: 7.02 ± 3.5 years). There was no statistically significant difference between the age and sex of patients ($P > 0.05$). Three cases (7%) had a history of brucellosis in their families and 88% ($N = 38$) reported a history of ingestion of raw or unpasteurized dairy products. Eleven patients (26%) had contact with suspicious animals. There was no statistically relevant association between the patients' gender and route of disease transmission ($P > 0.05$). All patients were Iranian, and the total number of reported patients in Tehran was higher than in other cities ($N = 12$, 28%).

Table 1 Demographic data of patients with brucellosis ($N = 43$)

Characteristics	No. of patients	%
Age, mean ± SD (years)	7.02 ± 3.5	–
Sex		
Male	26	60.5
Female	17	39.5
Family history of brucellosis	3	7
History of raw milk/dairy products consumption	38	88
Province		
Tehran	23	53.5
Alborz	7	16.3
West Azerbaijan	3	7
Kurdistan	1	2.3
Kerman	1	2.3
Khuzestan	1	2.3
Zahedan	1	2.3
Markazi	1	2.3
Semnan	1	2.3
Golestan	1	2.3
Ilam	1	2.3
Lorestan	1	2.3
Zanjan	1	2.3
History of contact with infected animals	11	26
Relapse	9	21

SD standard deviation

The lowest frequency of brucellosis was recorded in 2011 ($N = 2$, 5%) and the highest rates were observed in 2013 ($N = 10$, 23%) and 2015 ($N = 11$, 26%), (Table 2). Most cases were admitted in summer ($N = 14$, 33%) and spring ($N = 12$, 28%), and the lowest rate of cases was detected in winter ($N = 7$, 16%; Table 3).

Fever ($N = 39$, 91%), arthralgia ($N = 33$, 77%), and malaise ($N = 33$, 77%) were the main complaints and symptoms of patients, while only one patient (2%) had night sweating (Table 4). Thirty patients (70%) had the classic triad of undulating fever, sweating, and arthralgia. Other clinical findings included arthritis ($N = 22$, 51%), anorexia ($N = 19$, 44%), weight loss ($N = 16$, 37%), abdominal pain ($N = 11$, 26%), headache ($N = 7$, 16%), diarrhea ($N = 7$, 16%), splenomegaly ($N = 6$, 14%), vomiting ($N = 6$, 14%), cough ($N = 5$, 12%), and hepatomegaly ($N = 4$, 9%). Neurobrucellosis was seen in 2 patients (5%). One of these patients was a 9-year-old girl who was admitted to our hospital with a history of headaches and back pain existing for 1 year. Following a decrease of alertness, neuroimaging studies including head CT and MRI were performed. Tuberculosis was suspected, but the biopsy was negative for acid-fast bacilli. With suspicion of brucellosis, Wright, Coombs Wright, and

Table 2 Frequency of brucellosis cases in different years of the study period

Year	No. of patients	%
2011	2	5
2012	3	7
2013	10	23
2014	9	21
2015	11	26
2016	8	19
Total	43	100

Table 3 Frequency of brucellosis cases in different seasons of the year

Season	No. of patients	%
Spring	12	28
Summer	14	33
Autumn	10	23
Winter	7	16
Total	43	100

Table 4 Clinical manifestations of patients with brucellosis ($N = 43$)

Symptom/sign	No. of patients	%
Fever	39	91
Arthralgia	33	77
Malaise	33	77
Anorexia	19	44
Weight loss	16	37
Abdominal pain	11	26
Headache	7	16
Vomiting	6	14
Cough	5	12
Night Sweating	1	2
Splenomegaly	6	14
Hepatomegaly	4	9
Arthritis	22	51
Diarrhea	7	16

2 ME tests were performed. The 2 ME titer was 1:320, and Wright and Coombs Wright tests were 1:320 and 1:160, respectively.

The other case was a 13-year-old boy who was admitted with headache, vomiting, mild to moderate grade fever, and abdominal pain existing for 8 months. Paraclinical findings included hypereosinophilia in CBC (40%); 2 ME titer was 1:160 and Wright test was 1:320. With suspicion of brucellosis, treatment was initiated with rifampin, doxycycline, and gentamycin. In both cases, the blood and CSF cultures were negative and patients had a history of ingestion of raw or unpasteurized dairy products and contact with suspicious animals.

Table 5 shows hematological findings, the results of blood and bone marrow cultures, and results of serum agglutination tests of 43 children suffering from brucellosis. Leukocyte counts were normal in 33 patients (77%), 10 patients (23%) had leukocytosis, and no patients (0%) had leukopenia. Of all patients, 51% ($N = 22$) had lymphocytosis. The level of hemoglobin was normal in 15 patients (35%), whereas the remaining patients had anemia ($N = 28$, 65%). Thrombocytopenia was observed in 4 cases (9%). An elevated ESR was seen in 37 patients (86%) and elevated CRP values were recorded in 29 patients (67%). Increased levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were observed in 15 (35%) and 10 (23%) patients, respectively.

Blood culture was accomplished in 37 patients and positive in 11 patients (30%). Bone marrow culture was performed for only 13 patients and was positive in 4 cases (31%). Wright tests were performed for all patients, and 29 cases (67%) had an agglutination titer of 1/160 or higher. Coombs Wright and 2 ME tests were performed in 37 and 40 cases, respectively. Positive titers were observed in 34 cases (92%) in Coombs Wright and 34 cases (85%) in 2 ME. The highest titers for Wright and Coombs Wright were 1:2560 and 1:5120, respectively, and were observed in 1 patient. In 9 patients (21%) agglutination titers < 1:160 were recorded in the Wright test, but the titers were positive in Coombs Wright.

Patients received various antibiotic combination regimens that consisted of two- or three-drug therapy. The median length of antibiotic therapy was 12 weeks (range 2–24 weeks). In patients ≤ 8 years ($N = 27$, 63%), the following drug combinations were prescribed: trimethoprim-sulfamethoxazole and rifampicin ($N = 18$, 42%); trimethoprim-sulfamethoxazole, rifampicin, and gentamicin ($N = 6$, 14%); rifampicin and ciprofloxacin ($N = 2$, 4%); trimethoprim-sulfamethoxazole, rifampicin, and doxycycline ($N = 1$, 2%); and streptomycin and clarithromycin ($N = 1$, 2%).

In patients older than 8 years, six drug combinations were used: the combination of trimethoprim-sulfamethoxazole and rifampicin ($N = 6$, 14%); doxycycline and rifampicin ($N = 5$, 12%); trimethoprim-sulfamethoxazole, rifampicin, and gentamicin ($N = 1$, 2%); doxycycline, rifampicin, and gentamicin ($N = 1$, 2%), doxycycline, rifampicin, and streptomycin ($N = 1$, 2%); and trimethoprim-sulfamethoxazole, doxycycline, and streptomycin ($N = 1$, 2%).

Relapse occurred in 9 patients (21%), other patients did not experience recurrence of signs and symptoms of the disease. Of the patients with relapse, 44% (4 out of 9) were treated by the combination of trimethoprim-sulfamethoxazole and rifampicin; however, the difference between the type of treatment and rate of relapse was not statistically significant ($P > 0.05$). No deaths were observed among infected cases.

Discussion

Although the course of brucellosis is benign [2], if left untreated, it can become chronic and cause severe

Table 5 Laboratory findings in patients with brucellosis ($N = 43$)

Laboratory examination	No. of patients	%
Hematological manifestations		
Leucopenia (leukocyte count < 4000 cells/mm^3)	0	0
Leukocytosis (leukocyte count > 11,000 cells/mm^3)	10	23
Lymphocytosis (lymphocyte count > 40% of the total leukocytes)	22	51
Anemia (hemoglobin < 11 g/dL)	28	65
Thrombocytopenia (platelet count < 150,000 cells/mm^3)	4	9
Thrombocytosis (platelet count > 450,000 cells/mm^3)	0	0
Elevated ESR (ESR > 10 mm/h)	37	86
Elevated CRP (CRP > 6 mg/L)	29	67
Elevated ALT (>31 units/L for girls, >41 units/L for boys)	10	23
Elevated AST (>31 units/L for girls, >37 units/L for boys)	15	35
Elevated ALP (>1200 units/L)	0	0
Cultures		
Positive blood culture ($N = 37$)	11	30
Positive bone marrow culture ($N = 13$)	4	31
Agglutination tests		
Positive Wright test (titers > 1:160)	29	67
Positive Coombs Wright (titers > 1:40; $N = 37$)	34	92
Positive 2 ME (titers > 1:40; $N = 40$)	34	85

ESR erythrocyte sedimentation rate, *CRP* C-reactive protein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *2 ME* 2-mercaptoethanol

complications including endocarditis, meningoencephalitis, granulomatous hepatitis, uveitis, optic neuritis, pancytopenia, chronic anemia, spondylodiscitis, and sacroiliitis [9]. Despite control of brucellosis in the developed world, the disease remains an important health problem in developing countries [11] such as Iran, which is one of the main endemic areas [6]. Pediatric brucellosis is very important in Iran [18], rendering necessary the study of its clinical and epidemiologic characteristics in different regions of the country. This study describes the principal epidemiologic, clinical, and laboratory characteristics of children with brucellosis referred to the CMC Hospital in Tehran, Iran, between 2011 and 2016.

In the present study, the prevalence of brucellosis was 0.54%. In the earlier study conducted at this center from 2002 to 2010 [5], 34 out of 10,864 patients admitted to the infectious diseases ward (0.31%) were diagnosed as *Brucella*-infected cases.

In the current study, there was a male predominance among patients (60.5%), which is in agreement with the results of previous studies performed at the same hospital [5, 22] as well as other studies [1, 7, 9, 10, 15, 18, 23, 24]. However, in a study performed in Chicago, USA, most of the brucellosis cases were female [25]. Possible explanations for higher rates of brucellosis among boys could be the higher risk of direct contact with animals and consumption of unsafe food products in boys [5, 15, 26].

In this survey, brucellosis was more frequent in school-aged children (children ≥ 7 years of age) and none of the patients were younger than 1 year. This is in agreement with findings of other studies [1, 2, 18, 21, 23, 27]. However, in some studies, the infection was diagnosed in patients < 1 year of age [3, 7, 15]. The lower rate of infection in children younger than 1 year may be due to the fact that brucellosis has non-specific presentations or a milder course in this age group, which results in non-diagnosis and underreporting of infection in infants. In addition, breast milk may have anti-brucellosis antibodies that would prevent the occurrence of infection in infants [18].

In this study, 7% of subjects had a positive family history of brucellosis. This has also been reported in other studies [1, 7, 15, 18, 22]. The positive family history highlights the necessity of examining patients' family members for the presence of brucellosis.

In the current study, ingestion of raw unpasteurized milk or milk products was the main route of disease transmission (88%) and contact with suspected animals was in second place (26%), which is in accordance with the results of other studies [2, 5, 15, 18, 25, 28–30]. This reflects the importance of increasing awareness among both citizens and villagers about brucellosis transmission routes and the importance of avoiding consumption of unpasteurized raw milk and dairy products and contact with suspicious animals.

All patients in the present study were Iranian; none were migrants from other countries. In the Iranian study performed by Sasan et al. [7], only one child with brucellosis was an Afghan refugee and the remaining 81 patients were Iranian. Logan and colleagues from the USA [25] detected 22 cases of childhood brucellosis, with 19 cases (86%) having non-American nationalities. In addition, 18 of these cases

had recently traveled or resided in Mexico, another brucellosis endemic area. This indicates that in industrialized countries such as the USA, brucellosis is most commonly seen in migrants and travelers from endemic areas [7]. However, the abovementioned result confirms that brucellosis is endemic in the Iranian population and suggests the need for implementing preventive and control measures.

In the current study, brucellosis was more pronounced in summer and spring. A similar seasonal distribution was observed in previous studies performed at the CMC Hospital [5, 22] as well as in other studies from Iran [7] and Turkey [10, 24, 31]. The possible reason for this observation could be more livestock activities such as milking and slaughtering [15], increased access to fresh goat and sheep milk in local markets [7], a higher rate of travel to rural areas with consumption of fresh non-pasteurized dairy products in those areas [22], and consumption of traditional ice cream made from unpasteurized milk in spring and summer.

Brucellosis is a systemic disease, and *Brucella* can invade any organ. The variable symptoms are partly attributable to the variable pathogenicity of different strains of *Brucella* spp. [12]. Signs and symptoms of the patients in this study were consistent with those reported by other authors [3, 5, 18, 25, 28, 31], and fever, malaise, and arthralgia were the main complaints in brucellosis-infected children. Similar to other studies [28, 32], arthralgia (77%) was more common than arthritis (51%).

Liver function tests such as ALT and AST were elevated in 23 and 35% of cases, respectively, and hepatomegaly and splenomegaly were documented in 9% and 14% of patients, respectively. In the study conducted by Logan et al. [25], much higher rates of hepatomegaly (55%) and splenomegaly (60%) were noted. Various factors including duration of illness and quality of the examination can affect the reported rate of organomegaly in different studies [5].

Nonspecific gastrointestinal symptoms such as abdominal pain, diarrhea, and vomiting were observed in some patients. Mesenteric adenitis or inflammation and ulceration of the Peyer's patches induced by *Brucella* infection are the probable causes of these symptoms [28].

In the present study, anemia (65%), lymphocytosis (51%), and elevated ESR (86%) and CRP (67%) were the most prominent anomalies observed in *Brucella*-infected cases. Leukopenia, thrombocytosis, and pancytopenia were not detected in any case. In the previous study conducted at this center [5], anemia (53%) and leukopenia (33%) were the main hematological findings of patients and, similar to the current study, none of the patients had pancytopenia. In a study from Saudi Arabia [11] among 133 *Brucella*-infected children, anemia occurred in 43% of patients, followed by leukopenia (38%), leukocytosis (20%), and pancytopenia (18%). In the study conducted by Liang et al. from China [33], elevated CRP (71.1%) and ESR (63.2%) were the most frequent abnormal findings observed in 38 children with brucellosis. Several mechanisms contribute to anemia and other blood abnormalities associated with brucellosis, including hypersplenism [24, 28], bone marrow suppression, alteration of iron metabolism, autoimmune hemolysis [11, 24], hemophagocytosis, and granulomatous lesions of the bone marrow [11].

In this survey, blood culture and bone marrow culture were positive in 11 (N = 11/37, 30%) and 4 cases (4/13, 31%), respectively. In the study conducted by El-Koumi et al. [11], blood culture was positive in 23 out of 133 infected children (17%), and bone marrow culture was positive for 3 out of 9 tested children (33%). The low in vitro growth rate of *Brucella* spp. results in long incubation times and, therefore, a low bacterial isolation rate [13]. In addition, the diagnostic yield of blood culture differs in the acute, sub-acute, and chronic phases of the disease [4].

A positive titer in the Wright test (titers ≥ 1:160) is indicative of active brucellosis [24, 28] and the Coombs Wright test is performed to diagnose the sub-acute and chronic forms [28]. In this study, the Wright test was positive in 67% of patients. In nine cases (21%) the Wright test was negative, but the Coombs Wright was positive. In five patients (12%) the Wright test was negative, and disease was confirmed by positive Coombs Wright, 2ME, and blood or bone marrow cultures. The sensitivity of serological tests for diagnosis of brucellosis is between 65 and 95%; however, their specificity is lower. Factors influencing the specificity of these tests include the high prevalence of anti-brucellosis antibodies in the healthy population of endemic areas (a response to their continuous exposure to the infective sources [28]) and the occurrence of antigen cross-reactivity between various species of *Brucella* [4]. Therefore, in endemic areas, the serologic tests should be interpreted with regard to the clinical manifestations [12] and the diagnosis of brucellosis should not be based solely on these tests.

The main purpose of brucellosis treatment is to control the acute infection and inhibit the occurrence of disease relapse. Given the fact that *Brucella* is an intracellular pathogen, the selected drug regimens should have the ability to penetrate into cells [24, 28]. In addition, patient age is another factor influencing the selection of therapeutic regimens. According to World Health Organization (WHO) recommendations, the use of tetracyclines (such as doxycycline) in children < 8 years of age is prohibited, since they have side effects on children's teeth [3, 24]. Moreover, the use of quinolone-containing regimens is not recommended in children < 8 years of age [9]. The therapeutic regimens used in this study were highly variable, and the most frequent regimen was the combination of trimethoprim-sulfamethoxazole and rifampicin (N = 24, 56%). The median treatment

duration was 12 weeks (range 2–24 weeks). Various drug combinations and treatment durations for brucellosis have been reported in pediatric studies [3, 10, 21, 24, 25, 28]. According to the literature, it seems that combination therapy with two or three drugs active against *Brucella* spp. for a period of at least 6 weeks would be an effective treatment protocol for childhood brucellosis [21, 25].

In the present study, a relapse rate of 21% was observed. Relapse did not occur in patients who were treated with the following combinations: doxycycline, rifampicin, and streptomycin; trimethoprim-sulfamethoxazole, doxycycline, and streptomycin; doxycycline, rifampicin, and gentamicin; streptomycin and clarithromycin. In the previous study performed at this center [5], relapse was noted in 2.9% (1 out of 34) of patients. In the study conducted by Yoldas et al. [10] from Turkey, two patients (2%) experienced a relapse. Some of the main causes of brucellosis relapse include the occurrence of bacterial drug resistance [15], an inappropriate treatment regimen or inadequate treatment duration, a patient's failure to adhere to and complete the course of treatment, re-use of unpasteurized contaminated dairy products, and contact with infected livestock, especially in rural areas [7].

There are some limitations to this study. Firstly, it was conducted at the CMC Hospital, which is a tertiary children's referral hospital in Tehran, Iran, and patients from all over Iran refer to this hospital. Therefore, the patients did not comprise a representative sample of Tehran residence. Secondly, it was a single-center study and its study population was small. Thirdly, it was a retrospective study and results were extracted from medical records of the patients; where records were incomplete, we did not have access to the patients.

Conclusion

The findings of this study demonstrate that childhood brucellosis is an endemic disease in Iran. It is mostly related to consumption of unpasteurized dairy products and is more prevalent among children ≥ 7 years of age and male gender. Therefore, physicians should be aware of its manifestations, diagnosis, and treatment protocols. In addition, implementation of control programs and preventive measures by the government are highly recommended, such as regular examination of domestic animals, mass vaccination of livestock against *Brucella*, slaughter of infected animals, control of animal trade and migration, pasteurization of milk and milk products, as well as training and increasing public awareness about the dangers of consumption of unpasteurized dairy products.

Conflict of interest B. Pourakbari, M. Abdolsalehi, S. Mahmoudi, M. Banar, F. Masoumpour, and S. Mamishi declare that they have no competing interests.

References

1. Soleimani G. Evaluation of clinical findings and treatment of childhood brucellosis in Zahedan. Iran J Pediatrsoc. 2010;2:53–7.
2. Akbayram S, Dogan M, Akgun C, Peker E, Parlak M, Caksen H, et al. An analysis of children with brucellosis associated with pancytopenia. Pediatr Hematol Oncol. 2011;28(3):203–8.
3. Ahmetagić S, Porobić-Jahić H, Koluder N, Čalkić L, Mehanic S, Hadžic E, et al. Brucellosis in children in Bosnia and Herzegovina in the period 2000–2013. Med Glas. 2015;12(2):177–182.
4. Rubach MP, Halliday JE, Cleaveland S, Crump JA. Brucellosis in low-income and middle-income countries. Curr Opin Infect Dis. 2013;26(5):404.
5. Fanni F, Shahbaznejad L, Pourakbari B, Mahmoudi S, Mamishi S. Clinical manifestations, laboratory findings, and therapeutic regimen in hospitalized children with brucellosis in an Iranian Referral Children Medical Centre. J Health Popul Nutr. 2013;31(2):218.
6. Mirnejad R, Jazi FM, Mostafaei S, Sedighi M. Epidemiology of brucellosis in Iran: a comprehensive systematic review and meta-analysis study. Microb Pathog. 2017;109:239–247.
7. Sasan M-S, Nateghi M, Bonyadi B, Aelami M-H. Clinical features and long term prognosis of childhood brucellosis in northeast Iran. Iran J Pediatr. 2012;22(3):319.
8. Akhvlediani T, Clark DV, Chubabria G, Zenaishvili O, Hepburn MJ. The changing pattern of human brucellosis: clinical manifestations, epidemiology, and treatment outcomes over three decades in Georgia. Bmc Infect Dis. 2010;10(1):346.
9. Khalili M, Sharifi-Mood B, Metanat M, Salehi M. Epidemiology, clinical presentation, and treatment outcome of brucellosis among children. Int J Infect. 2014;1(3):e21570.
10. Yoldas T, Tezer H, Ozkaya-Parlakay A, Sayli TR. Clinical and laboratory findings of 97 pediatric brucellosis patients in central Turkey. J Microbiol Immunol Infect. 2015;48(4):446–9.
11. El-Koumi MA, Afify M, Al-Zahrani SH. A prospective study of brucellosis in children: relative frequency of pancytopenia. Mediterr J Hematol Infect Dis. 2013;5(1):.e2013011.
12. Alshaalan MA, Alalola SA, Almuneef MA, Albanyan EA, Balkhy HH, AlShahrani DA, et al. Brucellosis in children: Prevention, diagnosis and management guidelines for general pediatricians endorsed by the Saudi Pediatric Infectious Diseases Society (SPIDS). Int J Pediatr Adolesc Med. 2014;1(1):40–6.
13. Türel O, Sanli K, Hatipoğlu N, Aydoğmuş C, Hatipoğlu H, Siraneci R. Acute meningoencephalitis due to Brucella: case report and review of neurobrucellosis in children. Turk J Pediatr. 2010;52(4):426–9.
14. Zamani A, Kooraki S, Mohazab RA, Zamani N, Matloob R, Hayatbakhsh MR, et al. Epidemiological and clinical features of Brucella arthritis in 24 children. Ann Saudi Med. 2011;31(3):270.
15. Bosilkovski M, Krteva L, Caparoska S, Labacevski N, Petrovski M. Childhood brucellosis: review of 317 cases. Asian Pac J Trop Med. 2015;8(12):1027–32.
16. Zheng R, Xie S, Lu X, Sun L, Zhou Y, Zhang Y, et al. A systematic review and meta-analysis of epidemiology and

clinical manifestations of human brucellosis in China. Biomed Res Int. 2018;2018:5712920.
17. Lehman DC, Mahon C, Manuselis G. Textbook of diagnostic microbiology. Missouri: Saunders; 2015.
18. Ayazi P, Mahyar A, Rasoli A. Brucellosis in children. J Compr Pediatr. 2012;3(1):12–5.
19. Guven T, Ugurlu K, Ergonul O, Celikbas AK, Gok SE, Comoglu S, et al. Neurobrucellosis: clinical and diagnostic features. Clin Infect Dis. 2013;56(10):1407–12.
20. Nimri LF. Diagnosis of recent and relapsed cases of human brucellosis by PCR assay. Bmc Infect Dis. 2003;3(1):5.
21. Mantur B, Akki A, Mangalgi SS, Patil S, Gobbur R, Peerapur B. Childhood brucellosis—a microbiological, epidemiological and clinical study. J Trop Pediatr. 2004;50(3):153–7.
22. Afsharpaiman S, Mamishi S. Brucellosis: review of clinical and laboratory features and therapeutic regimens in 44 children. Acta Med Iran. 2008;46(6):489–94.
23. Parlak M, Akbayram S, Doğan M, Tuncer O, Bayram Y, Ceylan N, et al. Clinical manifestations and laboratory findings of 496 children with brucellosis in Van, Turkey. Pediatr Int. 2015;57(4):586–9.
24. Ulug M, Yaman Y, Yapici F, Can-Ulug N. Clinical and laboratory features, complications and treatment outcome of brucellosis in childhood and review of the literature. Turk J Pediatr. 2011;53(4):413.
25. Logan LK, Jacobs NM, McAuley JB, Weinstein RA, Anderson EJ. A multicenter retrospective study of childhood brucellosis in Chicago, Illinois from 1986 to 2008. Int J Infect Dis. 2011;15(12):e812–e7.
26. Dean AS, Crump L, Greter H, Hattendorf J, Schelling E, Zinsstag J. Clinical manifestations of human brucellosis: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2012;6(12):e1929.
27. Çaksen H, Arslan Ş, Öner AF, Cesur Y, Ceylan A, Ataş B, et al. Childhood brucellosis is still a severe problem in the eastern region of Turkey. Trop Doct. 2002;32(2):91–2.
28. Tanir G, Tufekci SB, Tuygun N. Presentation, complications, and treatment outcome of brucellosis in Turkish children. Pediatr Int. 2009;51(1):114–9.
29. Vafaii I, Ahangarzadeh Rezaee M, Abdinia B, Kolahdouzan K. Epidemiology, clinical manifestations, laboratory findings and outcome of brucellosis in hospitalized children in northwest of Iran. J Patient Saf Qual Improv. 2018;6(4):0.
30. Nabavi M, Hatami H, Jamali AH. Epidemiological, clinical and Paraclinical surveys of children and adolescents with brucellosis in Kohgiluyeh and Boyer Ahmad province in southwest of Iran during 2009–2015. Armaghane Danesh. 2018;23(2):236–52.
31. Buzgan T, Karahocagil MK, Irmak H, Baran AI, Karsen H, Evirgen O, et al. Clinical manifestations and complications in 1028 cases of brucellosis: a retrospective evaluation and review of the literature. Int J Infect Dis. 2010;14(6):e469–e78.
32. Sachdev A, Vohra R, Bijarnia S. Acute brucellosis of childhood: a case report with unusual features. 2001.
33. Liang C, Wei W, Liang X, Enjin D, Wang L, Bao M, et al. Clinical features of 38 children with brucellosis. Chin J Endem. 2017;36(8):590–2.