

Original Article

Presentation of Childhood Brucellosis in Western Greece

Ioannis Giannakopoulos, Nikoleta M. Nikolakopoulou, Maria Eliopoulou, Aikaterini Ellina, Febronia Kolonitsiou¹ and Dimitris A. Papanastasiou*

Department of Pediatrics and ¹Laboratory of Microbiology, School of Medicine, University of Patras, Patras, Greece

(Received November 18, 2005. Accepted March 28, 2006)

SUMMARY: The purpose of this study was to evaluate clinical and laboratory findings, treatment modalities and final outcomes of brucellosis in children and to compare our data with those of other studies performed in Greece. Fifty-two children treated for brucellosis in the Department of Pediatrics during the decade 1995-2004 were analyzed. Of the 52 children, 47 were reexamined during July 2005. Fever, arthritis or arthralgia, hepatomegaly and splenomegaly were the main findings. Young children had positive blood cultures and lower or negative antibody titers statistically significantly more often than did older children. *Brucella abortus* was isolated in 9 of 18 patients with positive blood cultures. Antibiotic treatment lasted for 28 days on average. There were no complications or relapses, except one, and the final outcomes were excellent.

INTRODUCTION

Brucellosis is a zoonosis with a worldwide distribution. It is an important infection of humans in many parts of the world such as Latin America, Southern Europe, Africa and Asia (1) and an occupational hazard in many countries such as the United States (2). The major reservoirs of the disease include goats and sheep (*Brucella melitensis*), swine (*B. suis*), cattle (*B. abortus*) and dogs (*B. canis*). Transmission to humans occurs mainly through consumption of infected animal products or direct contact with infected animals (2,3) and in rare instances from human-to-human contact (4,5). The clinical presentation of brucellosis is non-specific and heterogeneous in its course and severity (6), and in humans it presents as a multisystem disease (7,8) involving many organs and tissues. *Brucella* organisms are able to survive and even multiply within cells of the mononuclear-phagocytic system, thus explaining the tendency of the disease to have a prolonged clinical course with relapses and the difficulty in its treatment (9-11).

The aim of this study was to evaluate the clinical and laboratory findings, treatment modalities, complications and final outcomes of brucellosis in children in Western Greece. This is of interest because there are similar reports from other parts of this country (12,13) with demographic and epidemiologic characteristic different from those of Western Greece, and this study allowed us to compare the findings from these different areas. Furthermore, there has been a substantial increase in the relative incidence of childhood brucellosis in this country in the past two decades, although the incidence of the disease seems to have declined during that time period (14). In fact, during the mid 80s 7-8% of the total brucellosis cases occurred in children, whereas by the end of the 80s this percentage had risen to 12-13% (15). In support of these findings are the data of the last decade from the local Department of Health showing that of the

287 brucellosis cases reported, 69 patients, i.e., 24%, were children. Therefore, childhood brucellosis continues to be common in Greece, and knowledge about the disease as well as possible changes in its clinical course, complications and outcome remains of interest.

PATIENTS AND METHODS

During the 10-year period from 1995 to 2004, 52 children from Western Greece, aged ≤ 16 years and suffering from brucellosis were treated in our Department of Pediatrics. Of the 52 children, 47 were treated because of a first brucellosis attack that was diagnosed in our clinic, whereas the remaining 5 came to us for management of a relapse after initial oral treatment with trimethoprim/sulfamethoxazole in other institutions. Signs, symptoms, laboratory findings, treatment modalities, complications and final outcomes were retrospectively analyzed. Of the 52 children, 40 were reexamined during July 2005 in our department, 7 were contacted and interviewed by phone but were reexamined in other institutions during the same period, and 5 were lost to follow up. Reexamination included obtaining a history of the patient after discharge from the clinic until now, physical re-examination and an estimation of antibody titer using Wright's seroagglutination.

Laboratory diagnosis of brucellosis was based on a rapid slide screening test in which five serum sub dilutions were performed, followed in the case of positive results by the standard tube agglutination method (Immunistics, Inc., Ocean, N. J., USA). Titer 1:160 or greater, using continuous serum dilutions, was estimated as a positive result (12,16). Isolation of *Brucella* spp. from blood was performed by inoculating 3-5 ml of blood specimens into the BacT/Alert SA culture bottles incubated in the BacT/Alert Microbial Detection System for 3 weeks (bioMerieux SA, Lyon, France). In the case of positive results, subcultures of 0.5-ml samples were performed onto 5% blood and brucella agar plates (Difco Laboratories, Detroit, Mich., USA), incubated at 37°C for 24-48 h with and without CO₂ (16). Identification at the species level was based on carbon dioxide requirements for growth, biochemical tests, growth in the presence of thionine

*Corresponding author: Mailing address: Department of Pediatrics, School of Medicine, University of Patras, 26504 Patras, Greece. Tel: +30-2610-999545, Fax: +30-2610-994533, E-mail: dimapapa@med.upatras.gr

and basic fuchsin dyes and specific antisera for *B. abortus* and *B. melitensis* (Difco Laboratories) (16). Antibiotic susceptibility to rifampicin, doxycycline, sulfamethoxazole/trimethoprim, ceftazidime and ciprofloxacin was performed by the agar disk diffusion method (BBL, Becton Dickinson, Sparks, Md., USA).

Diagnosis of brucellosis was made either by isolating *Brucella* spp. from blood or by Wright's seroagglutination revealing a titer of antibodies to *Brucella* of $\geq 1:160$ apart from compatible clinical findings or both.

Data were statistically analyzed by using the χ^2 -test (with continuity correction), Mann Whitney U test, unpaired *t* test for comparison between groups and simple linear regression analysis. Statistical analysis was performed by application of the SPSS Version II statistical package.

RESULTS

The median age of the 52 children was 11.0 years (range from 0.08 to 16 years); 31 of them were males and 21 females. One male patient with congenital brucellosis (17) was 27 days old on admission. Only 17.3% (9/52) of the patients were ≤ 5 years, 25% (13/52) were older than 5 years but ≤ 10 years and 48.1% (25/52) were older than 10 years but ≤ 15 years. The remaining 5 patients were older than 15 years. Thirty-eight of the children were living in rural areas, 7 were living in suburban areas and the remaining 7 were living in urban areas. Land farmers or stock farmers were the parents of all children coming from rural areas and the parents of one child coming from a suburban area. All of the children except one were reported to have consumed unprocessed milk and milk products.

The mean time \pm standard deviation (SD) between onset of symptoms and admission to our hospital was 21 ± 19 days, range from 3 to 75 days. Table 1 demonstrates the main clinical symptoms and their frequency.

Fever was the most common symptom. Forty-two (81%) children presented with high fever, reaching 40°C , three of them with chills. Intermittent-undulant fever and night fever was observed in 11 and 8 children, respectively.

Arthralgia or arthritis involved mainly the large joints. It was observed in 43 (83%) children. One or both hips were affected in 19 (36.5%) patients, followed by the knees (5 children, 9.6%) and the ileosacral joints (3 children, 5.8%). Five children complained of pain in the lumbar region of the spine. Arthritis was documented in 6 patients, both clinically and by bone scan. Treatment cured all children with osteoarticular manifestations without complication or chronic involvement.

Table 1. Incidence and duration of clinical symptoms in the 52 children with brucellosis

Symptoms	No. of patients (%)	Mean duration of symptoms \pm SD in days (range)
Fever	42 (81)	9.9 ± 11.4 (0-45)
Arthralgia and arthritis	43 (83)	16.2 ± 16.3 (2-62)
Arthritis	6 (12)	
Weakness	10 (19)	11.8 ± 11.8 (2-40)
Sweating	8 (15)	10.9 ± 13.5 (2-45)
Myalgia	7 (14)	5.5 ± 4.9 (1-15)
Anorexia	7 (14)	3.3 ± 3.1 (1-10)
Weight loss	4 (8)	
Cough	4 (8)	12.8 ± 8.2 (8-25)
Headache	1 (2)	45

None of the patients had an exanthema related to the disease, except for 2 children with a maculopapular rash due to therapy.

On physical examination, hepatomegaly was found in 36 (69%) children, and it was the most common finding on admission. Splenomegaly, confirmed by ultrasonography, was detected in 25 (48%) patients. Lymphadenopathy, mainly of the cervical (13 patients) and lumbar (10 patients) regions, was present in 15 (29%) children. None of the children had signs or symptoms of central nervous system (CNS) disease.

Table 2 demonstrates the mean values of the laboratory findings. Wright's seroagglutination test was not performed initially in 4 patients with positive blood cultures. At least three titer estimations were done in each patient at different time intervals, except for the above 4 patients, in whom two estimations were done.

Table 3 demonstrates the initial values of antibody titers to brucella and the corresponding age of the patients. It is obvious that younger patients had lower titers than those of older patients. Simple regression analysis between titers of Wright's seroagglutination and ages showed a statistically significant effect of age on titers and confirmed that younger patients have lower titers than those of older patients ($r^2 = 0.18$, $F = 9.53$, $P = 0.0035$). There was no correlation between duration of symptoms before admission and brucella titers ($r^2 = 0.001$, $F = 0.031$, $P = 0.87$). Wright's seroagglutination test was negative in the patient with positive blood culture for *B. canis*. Three patients had titers less than 1:160, but all of them had positive blood cultures.

Six to 8 weeks after admission, a twofold reduction of the titer was detected in all patients with a titer $\geq 1:1,280$ and in 4 patients of 6 with a titer of 1: 640; in the remainder there was a titer decrease under 1:160. The mean duration of symptoms \pm SD at the time of the first Wright's seroagglutination was 16.3 ± 16.7 days (range 1 to 75 days). In 16 patients

Table 2. Laboratory findings in 52 children with brucellosis

Laboratory examination	Mean values \pm SD	Range
White blood cells (leucocytes/mm ³)	$7,405 \pm 3,055$	3,800-20,500
ESR (mm/h)	24 ± 17	2-85
AST (IU/L)	76 ± 70	12-315
ALT (IU/L)	71 ± 80	9-329

ESR, erythrocyte sedimentation rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 3. Mean age of brucellosis patients with different values of antibody titers

Values of antibody titers	Mean age \pm SD (years)	No. of patients (%)
1:1,280-2,560	11.3 ± 3.6	27 (51.9)
1:320-640	10.9 ± 3.6	17 (32.7)
1:40-160	4.9 ± 4.5	7 (13.4)

Table 4. Mean age and duration of symptoms in brucellosis patients with positive or negative blood cultures

Isolated bacterium	No. of patients	Mean age \pm SD (years)	Duration of symptoms
<i>B. melitensis</i>	8	7.4 ± 4.1	14 ± 8
<i>B. abortus</i>	9	9.3 ± 4.9	16 ± 15
<i>B. canis</i>	1	10.0	21
No bacterium	25	11.8 ± 3.7	20 ± 18

the duration of symptoms was shorter than 7 days, and in 9 patients the duration was 30 days or longer.

Blood cultures were performed in 43 of the 52 patients, and *Brucella* spp. were isolated in 18 (42%) of them (Table 4). No isolate was found to be resistant to the antibiotics tested, i.e., streptomycin, gentamicin, doxycycline, rifampicin and trimethoprim/sulfamethoxazole. Patients with positive blood cultures were significantly younger than those with negative blood cultures ($z = -2.437$, $P = 0.0148$). The mean age \pm SD of the patients with positive blood cultures was 8.5 ± 4.4 years, and that of patients with negative cultures was 11.8 ± 3.7 years. The duration of symptoms until admission did not significantly differ between patients with positive and negative blood cultures ($z = -0.250$, $P = 0.8029$).

The mean duration of treatment \pm SD was 28 ± 13 days (range 11 to 85 days). A shorter duration of treatment in a lot of patients was mainly due to reduced compliance during oral treatment. Forty-one children received parenteral therapy for a mean \pm SD of 14 ± 4 days (range 2 to 26 days). Thereafter, they continued oral therapy. The remaining 11 children received only per os therapy. One 2-year-old child relapsed. No complications of the disease, especially no occurrences of CNS disease, were detected during hospitalization of the children.

Forty of the 52 children were reevaluated during July 2005. None of them had a history of relapse after the completion of therapy. On clinical examination none had a palpable spleen or hepatomegaly, and all of them had a negative Wright's seroagglutination test. Seven children could not visit our institution; however, they reported no relapses and the Wright's seroagglutination reaction performed in other laboratories was negative. The remaining 5 children could not be reached for reevaluation.

DISCUSSION

Childhood brucellosis continues to be an important public health problem in our country, although a trend toward an overall decrease of the disease in Greece is suggested (14). However, this development is due mainly to a decline of brucellosis in adults and to a lesser degree in children (15), resulting in a relative increase in the frequency of the disease in children. Therefore, evaluation of the clinical features and laboratory findings of brucellosis in children remains of great importance.

In our study patients there is an obvious preponderance of males, and this is in accordance with other studies performed in Greece as well as in other countries (3,12,13,18). In agreement with other studies (3,12), all age groups were susceptible to the infection, but children older than 10 years were more often affected. Unprocessed milk and milk products are the main source of infection in nearly all cases, except a 27-day-old infant who presented with probably transplacentally transmitted congenital brucellosis (17). Other reports have also suggested that consumption of raw milk or dairy products is the main source of infection in children (3,12,13,18-21), whereas control of the disease in domestic animals and pasteurization of milk have led to the scarcity of childhood cases in the United Kingdom and the United States (22-24).

B. abortus and not *B. melitensis*, as expected, was the main pathogen isolated in the blood cultures. On the contrary, *B. melitensis* was almost the exclusive pathogen found in studies performed in other parts of this country, such as north-western (12,15) and central Greece (13). Also, in Middle

East countries such as Jordan, Iraq and Israel and in South Africa infections with *B. melitensis* predominate (3,18-21). *B. abortus* is more common in developed regions than *B. melitensis* and often leads to unapparent or mild illness (22,23).

The clinical manifestations in our series seem to be similar to those reported by others in Greece as well as in other countries (3,12,13,18-21). A combination of fever and arthralgia or arthritis were very common and of particular value for the diagnosis. In accordance with Al-Eissa et al. (3), it was found that arthritis or arthralgia involved most commonly the hip and knee joints and seldom or not at all the axial skeleton or small joints, in contrast to findings in adults (25,26). Involvement of the sacroiliac joint, a frequent manifestation in adult patients (27), was observed in only 5.8% of our patients.

Particular attention should be paid to the characteristics of fever, which have to be questioned in detail from the physician. The fever in brucellosis patients is often high, frequently accompanied by chills and, in a significant number of patients, the fever is undulant.

The most constant finding on physical examination was hepatomegaly, followed by splenomegaly and lymphadenopathy.

Young patients more often have positive blood cultures and lower antibody titers than do older ones. These findings probably indicate that young children have a reduced defence against *Brucella* pathogens compared to older children, which frequently leads to bacteraemia. Although none of the patients, even including those with positive blood cultures, had any complication, we conclude that brucellosis in young children may be more severe, and it requires particular attention because the risk for complications due to bacteraemia is increased. However, further confirmation of these findings is necessary, since we were not able to find other studies that could support our results. It is of interest that patients with brucellosis over 65 years have significant lower antibody titers than patients younger than 65 years of age (28).

Patients were treated with diverse therapeutic regimens and the treatment duration varied, since the study is a retrospective one. The mean duration of therapy was about 4 weeks and not the 6 weeks or longer that was proposed by the World Health Organization in 1986 to be the regimen of choice for brucellosis (29). However, only one child relapsed as a result of the short duration of treatment. It is well known that *B. abortus*, the main bacterial species causing infection in this series, provokes milder illness than does *B. melitensis*, and this may be the reason for the excellent treatment results and the lack of any complications in contrast to other studies in Greece as well as in other countries (3,12,13,18-21). However, excellent clinical outcomes despite the shorter duration of therapy could be due to a selection bias of the study, and therefore further data are needed to establish whether the shorter duration of antimicrobial therapy is adequate for childhood brucellosis due to *B. abortus*.

In the present study we attempted to evaluate clinical and laboratory findings, treatment modalities and final outcomes of brucellosis in children. It seems that better socioeconomic conditions and standards of living, compared to other parts of our country, result in milder illness, with no complications and better response to treatment.

REFERENCES

1. Salata, R. A. and Ravdin, J. I. (1985): *Brucella* species

- (brucellosis). p. 1283-1290. In Mandell, G. L., Douglas, R. G. and Bennet, J. E. (eds.), Principle and Practice of Infectious Diseases. 2nd ed. Wiley, New York.
2. Fox, M. D. and Kaufmann, A. F. (1977): Brucellosis in the United States, 1965-1974. *J. Infect. Dis.*, 136, 312-316.
 3. Al-Eissa, Y. A., Kambal, A. M., Al-Nasser, M. N., Al-Habib, S. A., Al-Fawaz, I. M. and Al-Zamil, F. A. (1990): Childhood brucellosis: a study of 102 cases. *Pediatr. Infect. Dis. J.*, 9, 74-79.
 4. Wood, E. E. (1955): Brucellosis as a hazard of blood transfusion. *Br. Med. J.*, I, 27-28.
 5. Naparstek, E., Block, C. S. and Slavin, S. (1983): Transmission of brucellosis by bone marrow transplantation. *Lancet*, 1, 773.
 6. Colmenero, J. D., Reguera, J. M., Martos, F., Sanchez-De-Mora, D., Delgado, M., Causse, M., Martin-Farfan, A. and Juarez, C. (1996): Complications associated with *Brucella melitensis* infection: a study of 530 cases. *Medicine (Baltimore)*, 75, 195-211.
 7. Matyas, Z. and Fujikura, T. (1984): Brucellosis as a world problem. *Dev. Biol. Stand.*, 56, 3-20.
 8. Young, E. J. (1995): An overview of human brucellosis. *Clin. Infect. Dis.*, 21, 283-290.
 9. Caning, P. C., Roth, J. A. and Deyoe, B. L. (1986): Release of 5'-guanosine monophosphate and adenine by *Brucella abortus* and their role in intracellular survival of the bacteria. *J. Infect. Dis.*, 154, 464-470.
 10. Ocon, P., Reguera, J. M., Morata, P., Juarez, C., Alonso, A. and Colmenero, J. D. (1994): Phagocyte cell function in active brucellosis. *Infect. Immun.*, 62, 910-914.
 11. Pellicer, T., Ariza, J., Foz, A., Pallares, R. and Gudiol, F. (1988): Specific antibodies detected during relapses of human brucellosis. *J. Infect. Dis.*, 157, 918-924.
 12. Galanakis, E., Bourantas, K. L., Leveidiotou, S. and Lapatsanis, P. D. (1996): Childhood brucellosis in northwestern Greece: a retrospective analysis. *Eur. J. Pediatr.*, 155, 1-6.
 13. Tsolia, M., Drakonaki, S., Messaritaki, A., Farmakakis, T., Kostaki, M., Tsapra, H. and Karpathios, T. (2002): Clinical features, complications and treatment outcome of childhood brucellosis in central Greece. *J. Infect.*, 44, 257-262.
 14. Manetas, S., Tselentis, Y. and Charvalos, A. (1989): Epidemiological investigation of human brucellosis in Greece. *Ital. J. Epidemiol.*, 6, 393-396.
 15. Galanakis, E., Kefallinou, A., Kostoula-Tsiara, A. and Lapatsanis, P. D. (1993): Childhood brucellosis in Epirus (NW Greece) during the decade 1980-89. *Paediatr. Dis.*, 56, 162-168.
 16. Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C. and Winn, W. C. (1992): *Brucella* species in Diagnostic Microbiology. 4th ed. Lippincott Co., Philadelphia, USA.
 17. Giannakopoulos, I., Eliopoulou, M. I., Ziambaras, T. and Papanastasiou, D. A. (2002): Transplacentally transmitted congenital brucellosis due to *Brucella abortus*. *J. Infect.*, 45, 209-210.
 18. Al-Dubooni, H. M., Al-Shirkat, S. A. and Nagi, N. A. (1986): Brucellosis in children in Iraq. *Ann. Trop. Paediatr.*, 6, 271-274.
 19. Gottesman, G., Vanunu, D., Maayan, M. C., Lang, R., Uziel, Y., Sagi, H. and Wolach, B. (1996): Childhood brucellosis in Israel. *Pediatr. Infect. Dis. J.*, 15, 610-615.
 20. Benjamin, B. and Annobil, S. H. (1992): Childhood brucellosis in southwestern Saudi Arabia: a 5-year experience. *J. Trop. Pediatr.*, 38, 167-172.
 21. Hendricks, M. K., Perez, E. M., Burger, P. G. and Mouton, P. A. (1995): Brucellosis in childhood in the Western Cape. *S. Afr. Med. J.*, 85, 176-178.
 22. Spink, W. W. (1956): The nature of brucellosis. Minneapolis, MN: University of Minnesota Press.
 23. Street, L., Grant, W. W. and Alva, J. D. (1975): Brucellosis in childhood. *Pediatrics*, 55, 416-421.
 24. Bothwell, P. W. (1982): Brucellosis in children. *Arch. Dis. Child.*, 37, 628-639.
 25. Solera, J., Lozano, E., Martinez-Alfaro, E., Espinosa, A., Castillejos, M. L. and Abad, L. (1999): Brucellar spondylitis: review of 35 cases and literature survey. *Clin. Infect. Dis.*, 29, 1440-1449.
 26. Ayatollahi, J. (2004): Epidemiological, clinical, diagnostic and therapeutic survey of 686 cases of brucellosis. *Ann. Saudi. Med.*, 24, 398-399.
 27. Benjamin, B., Annobil, S. H. and Khan, M. R. H. (1992): Osteoarticular complications of childhood brucellosis: a study of 57 cases in Saudi Arabia. *J. Pediatr. Orthop.*, 12, 801-805.
 28. Colmenero, J. D., Reguera, J. M., Cabrera, F. P., Cisneros, J. M., Orjuela, D. L. and Fernandez-Crehuet, J. (1990): Serology, clinical manifestations and treatment of brucellosis in different age groups. *Infection*, 18, 152-156.
 29. Joint Food and Agriculture Organization (1986): FAO/WHO Expert Committee on brucellosis (sixth report). WHO Technical Report Series. no. 740. p. 56-57. World Health Organization, Geneva.