

Cat-scratch disease in Crete: an update

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Abstract

There are few epidemiological and clinical studies about the presence of cat scratch disease (CSD) on the island of Crete. The objective of this study was to analyze a large number of patients with suspected CSD to define the frequency of *Bartonella* infections in Crete. From January 2005 to October 2008, we studied patients with suspected CSD from hospitals in Crete. Sera of the referred patients were tested by immunofluorescence assay (IFA). For some patients, we also received lymph nodes and blood samples that we tested for the presence of *Bartonella henselae* by molecular assays. Overall, we tested 507 serum samples and we found 56 (11%) cases of CSD. PCR assay was positive for 2 patients; one had a *B. henselae* positive lymph node and the other a positive whole blood sample. Significantly more CSD cases (62.5%, 35 of 56) were reported in children than in infants and adults ($P < 0.05$). Moreover, we identified that most cases of CSD occurred between May and September ($P = 0.002$) and December and January. CSD is prevalent in Crete and is mostly associated with an increase in outdoor activity.

Introduction

Bartonella henselae is the main causative agent of cat scratch disease (CSD), which appears to be the most common organism responsible for lymphadenopathy in adults and children.¹ Recently, *B. henselae* was also associated with a syndrome characterized by scalp eschar and neck lymphadenopathy following tick bites (SENLAT).² Rarely, CSD has also been associated with *B. quintana* and recently with *B. alsatica*.³ Cats are the main reservoirs of *B. henselae*, and the bacterium is transmitted among cats by the cat flea (*Ctenocephalides felis*).¹ However, epidemiological data do not support efficient transmis-

sion from cat to human via the *Ct. felis*⁴ and most case series of CSD patients report that symptoms developed after the patient received a scratch.⁵ The transmission of *B. henselae* from cat to human via a scratch is nevertheless a rare event, considering the number of pet cats and the frequency of cat scratches and bites inflicted by them.

Patients with classic CSD usually present a gradual regional lymph node enlargement, usually accompanied by a round, red-brown, non-tender papule, which develops in the line of the scratch after 3-10 days and may persist for only a few days or as long as 2-3 weeks.⁶ Recently, live *B. henselae* was found in the primary inoculation sites of 3 patients after a cat scratch.⁷ The enlarged lymph node is often painful and tender.⁶ Lymphadenopathy occurs in the nodes that drain the area where cat scratches usually occur; in the axilla, the neck, and the groin.⁸ The infection is usually self-limited, with the frequent development of extensive regional lymph node enlargement that typically lasts 2-3 months and occasionally longer.⁶ Most patients with typical CSD remain afebrile and are not systemically ill throughout the course of the disease. As a reference center for bartonellosis, we routinely receive samples from patients with suspected CSD. In this study, we analyzed a large number of patients with suspected CSD in Crete during a 3-year period. Our objective was to define the frequency of *Bartonella* infections and to characterize clinical and laboratory features.

Materials and Methods

Patients

From January 2005 to October 2008, we studied patients with suspected CSD from hospitals of the four prefectures of Crete: Chania (30 patients), Rethymno,¹⁴ Heraklion (446), and Lasithi.¹⁷ Clinico-epidemiological patient inclusion criteria were: fever over 38°C and/or lymphadenopathy and/or visual disturbance and/or contact with cat or fleas. A standardized questionnaire to collect this information, together with details of treatment, outcome, possible complications, etc., was completed for each patient. For each patient, we received at least one serum sample. For 122 patients we also received a convalescent-phase serum sample. For 10 patients, we also received a lymph node biopsy specimen and a whole blood sample. Patients were classified as definitely having CSD if there was direct evidence of infection with *Bartonella* sp. using culture, serology or molecular assays.

Serology

Sera of the referred patients were tested by

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immunofluorescence assay (IFA) for IgM and IgG antibodies against *B. henselae* (FOCUS, USA) according to the manufacturer's recommendations. Sera were considered positive in cases of: a) a 4-fold increase in IgM and/or IgG antibodies in two consecutive samples; or b) titers of IgM antibodies over 1/80 and or titers of IgG antibodies over 1/512 in a single sample.

PCR assays

DNA was extracted using a QIAamp tissue kit (QIAGEN, Hilden, Germany). DNA samples were stored at -20°C until further processing. PCR amplifications were carried out using the primer pair BartoITSF and BartoITSR targeting the intergenic spacer region of *Bartonella* spp.⁹ Up to 10 samples were tested along with negative controls (DNA from non-infected lymph nodes and sterile water) and a positive control (DNA from *B. elizabethae*).

Culture

Lymph nodes and blood samples were cultured on blood Columbia agar plates, incubated at 37°C in an atmosphere of 5% CO₂, and examined weekly for growth during a 2-month period, as previously described.¹⁰

Statistical analysis

Data were analyzed using the SPSS v.16 software. ANOVA and Student's t-test were used taking $P \leq 0.05$ to be significant.

Results

Overall, we tested 507 serum samples and we found 56 (11%) cases of CSD (Table 1). In 15 patients a different diagnosis was made other than CSD. *Bartonella henselae* cross reactions with other microorganisms or even with cancer, lymphoma etc. may occasionally be misleading. By IFA, *Bartonella* positive results were obtained for 54 (10.6%) patients. PCR assay was positive for 2 patients; one had a *B. henselae* positive PCR in lymph node and the other a positive PCR in the whole blood sample. For both these 2 patients IFA was negative. No isolates were obtained from the culture of the lymph nodes and blood samples.

Eight (14.2%) CSD cases were recorded in 2005, 13 (23.2%) in 2006, 20 (35.7%) in 2008 and 15 (26.7%) in 2008. Positive CSD cases were plotted for each month (Figure 1) to identify seasonal distributions of CSD from 2005 through 2008. Monthly mean numbers of CSD were lowest from February through April, followed by significant increases during May to September ($P=0.002$) and during December to January ($P=0.01$). The age distribution of the 56 confirmed cases ranged from nine months to 73 years. Ages were categorized according to the official age distribution: infants, 0-3 years; children, 4-15 years; adults, over 15 years (Figure 1). Significantly more CSD cases (62.5%, 35 of 56) were reported in children than in infants and adults ($P<0.05$). No statistically significant difference was calculated in terms of gender (male 42.6%, female 57.1%).

Epidemiological data revealed that contact with cats was reported in 11 (19.6%) of the confirmed cases. The clinical features of CSD were local lymphadenopathy (76.8%) and fever (71.4%) usually persisting for a few days. In 13 cases (23.2%) lymphadenopathy without the presence of fever was recorded. Other symptoms such as headache, malaise, arthralgias, myalgias, anorexia and vomiting were reported less often (Table 2). Twenty (35.7%) patients presented slight anemia (Hb <12 g/dL) on hospitalization. Leukocytosis was recorded in 15 (37.5%) patients, with levels ranging from $11 \times 10^9/L$ to $23 \times 10^9/L$. Platelet levels were within normal range in all patients. Complications due to infection by *B. henselae* were observed in 4 patients. Of those, 2 women suffered from sudden vision loss without the co-existence of other clinical symptoms. Vision was restored post treatment. One patient suffered from enlargement of liver and spleen of up to 1.5 cm together with enlargement of the mesothoracic lymph nodes, all of which receded upon drug therapy. A 1.5 year old infant developed pneumonia together with encephalopathy, hydrocephalus and Guillan Barre syndrome.¹¹

Forty patients (71.4%) received antibiotic

therapy; 13 patients (15.7%) were treated with ciprofloxacin, 12 patients (15%) with chloramphenicol and one (1.8%) with doxycycline. Additionally, 9 patients (10.8%) received a combination of doxycycline and chloramphenicol, while 23 patients (27.7%) received doxycycline with ciprofloxacin. All patients receiving antibiotic treatment fully recovered.

Discussion

In a previous seroepidemiological study conducted in 491 samples of healthy blood donors in Crete, a 21.6% seroprevalence against *B. henselae* was recorded.¹² In the current study, we identified 56 cases of CSD during a 3-year period. Our results were based on clinical and epidemiological data and on molecular and

serological assays. Our PCR assay had been previously validated for the diagnosis of CSD in lymph nodes biopsies.^{1,9} Although culture of lymph nodes and blood samples was systematically performed, we did not obtain *Bartonella* isolates. Indeed, *B. henselae* is not frequently grown from human lymph nodes and only in a few cases has *B. henselae* been isolated from patients with CSD.^{1,13}

Most of our cases were children. This agrees with other studies that have shown that most cases of CSD occur in children and young adults.¹⁴ However, a small number of patients with CSD aged over 60 years were also identified and characterized. In a previous study in Israel, Ben Ami *et al.* found that CSD affects elderly subjects as well as other age groups.¹⁵ In this study, the most cases were recorded during the summer period with another peak recorded in winter. In the USA, it was found that CSD

Table 1. Distribution of IgG titers in each age group.

Titer of IgG antibodies	Age (years)			
	0-20	21-30	31-40	≥ 51
0	14	0	1	0
1/64	1	0	0	0
1/128	5	0	0	0
1/256	6	0	0	0
1/512	13	4	2	1
1/1024	6	0	1	1
1/2048	1	0	0	0

Table 2. Signs and symptoms recorded among the 56 confirmed Cat-scratch disease cases.

Signs – symptoms	No	%
Fever	40	71,4
Lymphadenopathy	43	76,8
Neck	20	35,7
Inguinal	11	19,6
Armpit	12	21,4
Hepato-splenomegaly	7	12,5
Arthralgia	5	8,9
Malaise	5	8,9
Rash	5	8,9
Maculopapular	1	1,8
Papular	1	1,8
Erythema nodosum	2	3,6
Headache	4	7,1
Impaired vision (vision loss, squint)	8	14,3
Myalgia	2	3,6
Anorexia	3	5,3
Vomiting	2	3,6
Pericarditis	2	3,8
Arrhythmias	5	8,9
Confusion, loss of conscience	6	10,7
Hematuria	2	3,5
Stiff neck	2	3,6

presented a seasonal variation from September through January¹⁶ and most CSD-associated hospitalizations for patients under the age of 18 years occurred between July and October.¹⁷ In addition, in Japan, CSD mainly occurred in the fall and winter months, as most cases occurred between September and December.¹⁸ In France, a reduced incidence was recorded during spring with an increase in autumn and peaks in December.¹⁹ The difference observed in the United States may be attributed to the fact that it is a large country with diverse climates. As far as Crete is concerned, the fact that cats have three reproduction periods rather than two may well explain the presence of the peak recorded during winter.

People in Crete do not keep pets like cats indoors and probably the increase of CSD cases during the summer is explained by the increase in outdoor activity and greater contact with pound or street cats. Moreover, the higher seroprevalence of *B. henselae* in the pet cat population in warm, humid climates than in cats in cold, dry climates is explained

because *Ct. felis* is more common in warmer climates.²⁰ This can be explained because the environmental conditions of temperature and relative humidity are the two most important factors for the successful reproduction, development and survival of fleas.²¹ As a result, cats present more fleas during the summer.²²

Local lymphadenopathy and fever were the most common symptoms in our study. Uncommon clinical signs were malaise, fatigue, myalgia and arthralgia, and hepatosplenomegaly. In patients with classic CSD, lymphadenopathy is the most common symptom and occurs most frequently in the axillary and epitrochlear nodes (46%), head and neck (26%), and the groin (17.5%).²³ Most patients with typical CSD remain afebrile and in a series of 1,200 cases the disease was considered mild in over 75%; 41% were afebrile, 31% had fever below 39°C, 19% had fever by touch and only 9% had fever over 39°C.²⁴ In another series of 1,722 patients with CSD, almost one-half had lymphadenopathy only.²⁵ Systemic or severe disease can complicate

CSD in 5-14% of cases.²³ Atypical presentations include prolonged fever (over 2 weeks), malaise, fatigue, myalgia and arthralgia, weight loss, splenomegaly, and Parinaud's oculoglandular syndrome.²⁶ Encephalopathy and neuroretinitis are less common complications of CSD.²⁶

In conclusion, we proved that CSD is prevalent in Crete and is seasonal starting in May and finishing in September. The increase in outdoor activity and in *Ct. felis* during the summer months probably explain this seasonality. The differential diagnosis of CSD is often misleading and more serious conditions (mycobacterial or fungal infections, lymphoma, melanoblastoma, carcinoma) are often considered. These may entail unnecessary, expensive, and often invasive evaluations and protracted hospital stays. Because of this, accurate diagnosis is of critical importance. Physicians need to inquire about recent contact with an animal, especially felines, or injury from an animal, when a patient presents with or without lymphadenopathy.

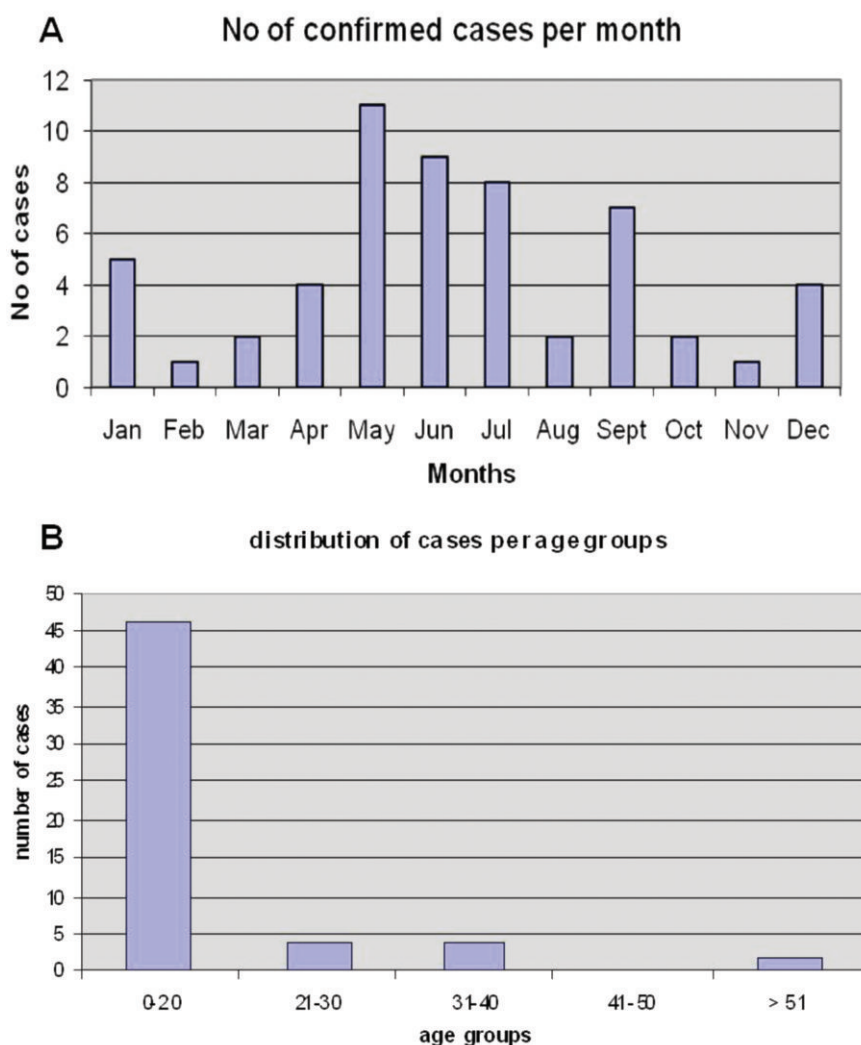


Figure 1. Distribution of Cat-scratch disease cases by (A) month and (B) age.

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