UPDATE ARTICLE

ARTICOLO DI AGGIORNAMENTO

Clinical features and treatment of Mediterranean Spotted Fever in children: a practical update for clinicians

Manifestazioni cliniche e terapia della febbre bottonosa del Mediterraneo: un pratico aggiornamento per il clinico

A. CASCIO, C. IARIA
AILMI (Associazione Italiana per la Lotta contro le Malattie Infettive) c/o Scuola di Specializzazione in Malattie Infettive, Università di Messina

Summary

Mediterranean spotted fever (MSF) is a tick-borne disease caused by *Rickettsia conorii.* It is characterized by the symptomatologic triad of fever, exanthema and «tache noire» – the typical eschar at the site of the tick bite. Oral or parenteral administration of tetracyclines or chloramphenicol represent the standard treatment; however, both these drugs may cause significant adverse effects in children. Recent studies indicate that oral clarithromycin and azithromycin may represent an acceptable alternative for the treatment of children with MSF. There are no data to indicate that antimicrobial prophylaxis is beneficial for tick-bitten patients to prevent MSF. However, in the presence of a lesion ascribed to «tache noire», antibiotic treatment should be started even in the absence of fever or exanthema.

Riassunto

La Febbre bottonosa del Mediterraneo (FBM) è una rickettsiosi trasmessa da zecche causata da Rickettsia conorii. La malattia è caratterizzata dalla triade sintomatologica: febbre, esantema e «tache noire», la tipica escara necrotica che compare nella sede della puntura della zecca. Il trattamento ha previsto finora l'utilizzo per via orale o parenterale del cloramfenicolo o delle tetracicline, farmaci che possono causare gravi effetti collaterali nel bambino. Studi recenti indicano che l'azitromicina e la claritromicina somministrati per via orale possono rappresentare una valida alternativa nel trattamento della FBM in età pediatrica. Non ci sono dati che indicano che la chemioprofilassi nei soggetti punti dalla zecca è utile per prevenire la FBM. Comunque in presenza di una lesione ascrivibile alla «tache noire» la terapia antibiotica dovrebbe essere iniziata anche in assenza di febbre ed esantema.

Key words

Spotted Fever • Boutonneuse fever • Rickettsia • Clarithromycin • Azithromycin

Parole chiave

Febbre bottonosa • Rickettsia • Zecca • Claritromicina • Azitromicina

Submitted: November 21, 2003

Correspondence:
Prof. Antonio Cascio
AILMI (Associazione Italiana per la Lotta contro le Malattie Infettive)
c/o Scuola di Specializzazione in Malattie Infettive
Università di Messina
Via Consolare Valeria 1
98125 Messina
Italia.
Tel. +39 090 221 2033
Fax +39 090 2939512
E-mail: acascio@unime.it

Introduction

Mediterranean spotted fever (MSF) (or Boutonneuse fever) is a tick-borne disease caused by *Rickettsia conorii*. In the Mediterranean area this organism is transmitted to humans by the brown dog tick *Rhipicephalus sanguineus* (in the nymph, larvae or adult stage), generally considered to be the most common tick present in this region [1-3] (Fig. 1).

However, *R. conorii* has been documented in at least 25 other species of tick of the Ixodidae family. Some of these parasites are small mammals and could play a role in ensuring the wild cycle of this rickettsia [4]. Ticks are the only vector and the main host of *R. conorii*, being rickettsiae transmitted vertically and transovarially from females via the eggs to the larvae of the next generation [3-6] (Fig. 2).

Dogs can be infected by *R. conorii*, but clinical signs of the disease have not been reported [7]. By acting as natural hosts for *R. sanguineus*, dogs significantly increase contact between these species and humans, thereby increasing the risk of transmission [8].

Fig. 1. *Rhipicephalus sanguineus* on vegetation waiting for passing hosts, with its front legs held out in an ambush strategy.

Ixodid ticks (or «hard ticks», thus called for their sclerotized dorsal plate) spend > 90% of their life unattached from the host. They are usually seasonally active, seeking their hosts when environmental conditions are most suitable. They are highly responsive to stimuli that indicate the presence of hosts. These include chemical stimuli (such as CO_2, NH_3) phenols, humidity, aromatic chemicals, airborne vibrations and body temperature associated with warm-blooded animals. In their ambush strategy, ticks climb up vegetation and wait for passing hosts, with their front legs held out in the same manner as are insect antennae [3]. Many ticks lack eyes, and even when eyes are present, they are unlikely to enable a detailed perception of the environment. However, ticks have a variety of peripheral sensory organs. These include hair-like structures on the body, legs, and mouthparts, and a sensory complex located on the dorsal surface of the tarsus of leg I, which contains a cluster of olfactory and gustatory receptors (Haller's organ). These sensory organs are clearly important in enabling ticks to locate their hosts [3].

Interestingly, the number of cases of MSF in Italy and elsewhere appears to have increased over the past 20 years [9-10] (Fig. 3). Sporadic cases as a result of tourism have been diagnosed in other countries [11] and in North America, where MSF is the most frequently imported rickettsiosis [12]. Many factors have been called into play to explain the increase in the number of cases [4] (Tab. I). In Italy the regions with the highest incidence are Sicily and Sardinia; in 1998, an average of 8.8 cases occurred every 100,000 people in Sicily, compared with the national average of 1.6 cases per 100,000 people. The seasonal peak of the disease (June through September) occurs during maximal activity of immature stage ticks [13 14] (Fig. 4).

Clinical features

The infecting tick bite is painless, and the tick is usually unnoticed, especially in the case of smaller larvae or nymphs . When not engorged, at these stages ticks are smaller than a pinhead. A history of tick bite is an important finding but is often absent. In several cases of MSF, ticks were found at the site of a bite on patients who had been ill for several days. The patients simply had not noticed the presence of ticks, which must have been attached for more than 10 days, since the incubation period for the disease is usually 7 days [15 16].

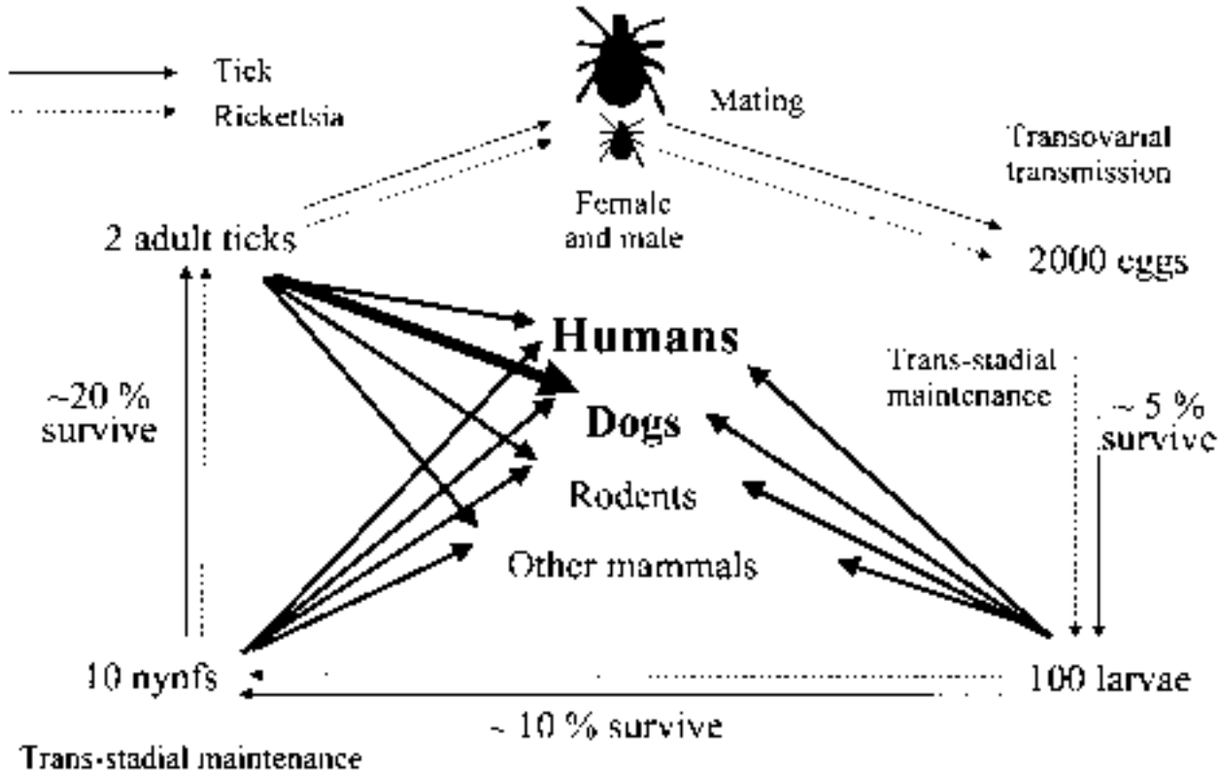
MSF is characterized by a symptomatologic triad: fever, exanthema and «tache noire». The onset of fever is generally sudden, and in typical cases body temperature is > 39 °C. Usually exanthema ensues 2-3 days after the fever and rarely later than the 5th day. Exanthema is initially macular and sparse and generally appears first on the wrists and ankles, spreading rapidly to the palms and soles (Fig. 5); it subsequently becomes maculopapular and extends to the trunk and less frequently to the face, often turning red-purple in color. In some patients only a few lesions resembling mosquito bites may be present, in others the exanthema may be petechial or purpuric and rarely papulovesicular [1]. While papulovesicular exanthema seen in MSF cases may be explained by variant strains of *R. conorii* or, alternatively, by an unusual host response to the infection, it cannot be excluded that it might be caused by other *Rickettsia* species, and serologically "positive" results may be due to cross-reactions. For example, *R. akari*, the agent of rickettsialpox, is a possible cause of papulo-vesicular exanthema. While this organism has never been isolated in Italy, it has recently been reported in Croatia [15].

The «tache noire», the typical eschar at the site of the tick bite, is present in about 70% of cases (Fig. 6). It is generally – but not necessarily – a single lesion (Fig. 7); the most common localizations of tache noire seen in a large series are shown in Figure 8. On the scalp the «tache noire» is often surrounded by an area of alopecia (Fig. 9). Lymph nodes draining the skin site of the tick bite can be tender and slightly painful on palpation. Arthralgia and/or myalgia generally involve the joints and the muscles of the lower limbs, but only rarely do they restrict mobility. Arthritis generally affects a single joint of the lower limb with moderate signs of inflammation (slight tenderness, erythema, and swelling and pain on motion). Arthralgia is more severe in adults. Headache is a rare complaint in children, while it is a typical symptom of the disease in adults [2].

Non-exanthematic forms can occur, and, in these cases, the only signs of infection can be the presence of lymphadenopathy and/or tache noire and/or fever [1]. Non-exanthematic forms may at least in part explain the discrepancy between the high prevalence of seropositivity and the prevalence of the disease documented in some studies [10].

In the Western Hemisphere, the Rocky Mountain spotted fever (RMSF), caused by *Rickettsia rickettsii*, can be a severe disease, but MSF is generally milder. Historical studies have shown that MSF can lead to 10-14 days of fever if untreated, and that it is rarely fatal in children [2]. Severe forms of the disease have been reported in 6% of patients, especially adults with one of the following conditions: diabetes, cardiac disease, chronic alco-

Fig. 2. Life cycle of *Rhipicephalus sanguineus* and *Rickettsia conorii*.



The life cycle of ixodid ticks is a 3-host one, with a single host chosen at each feeding stage of the tick (larva, nymph, and adult). At each developmental stage the tick searches for a host, attaches to it, and then feeds over a period of several days. Once replete, the tick drops off the host and looks for a place where it can digest its blood meal. The tick can then moult to the next stage, or enter diapause, a condition in which metabolism is reduced and development delayed [3].

The life cycle is usually completed in 2-3 years, but may take from 6 months to 6 years, depending on environmental conditions, including temperature, relative humidity, and photoperiod. Mating generally occurs on the host. Gravid females lay thousands of eggs on the grass (4-6 weeks). Eggs hatch into larvae (4-6 weeks); next, engorged larvae shelter in grass and moult into nymphs (4-6 weeks); finally, engorged nymphs shelter in the grass and moult into adults (10-20 weeks) [3]. The transmission cycle of *R. conorii* is shown in the dashed circle. Infection with *R. conorii* can induce in *R. sanguineus* mortality, severe malformations and alterations in the feeding behavior (delayed engorgement) [6].

The adult stage of the brown dog tick *Rhipicephalus sanguineus* is very host-specific; it rarely bites humans [13]. Larval and nymph stages are usually less specific in their choice of the host and bite humans more often than adult ticks do.

Before feeding, a tick may wander around on its host for several hours. It inserts only its hypostome into the skin, inoculating the host with various substances produced by the salivary glands, creating a feeding pool. During the first 24-36 h of attachment, there is little or no ingestion of blood.

The salivary secretions produced by ixodid ticks include a kind of cement to anchor the mouthparts to the skin of the host; enzymes; vasodilators; and anti-inflammatory, antihemostatic, and immunosuppressive substances. These facilitate successful blood feeding, and an anesthetic in the saliva makes the bite of Ixodid ticks usually painless [3].

The feeding period of ixodid ticks is quite long, requiring 2-15 days for a complete blood meal to be ingested; the length of the ingestion period depends on different factors, such as the species of tick, the feeding stage, the type of host, and attachment site. After an initial slow feeding phase (3-4 days) a period of rapid engorgement follows (1-3 days) during which the body weight of ticks, particularly females, may increase up to 120-fold. While feeding, blood sucking and salivation alternate and, at the end of the rapid engorgement phase, regurgitation occurs frequently, thus increasing the transmission of infectious agents.

holism, glucose-6-phosphate dehydrogenase deficiency, end stage kidney disease. Mortality rate may reach 2.5%. Patients with malignant forms have a petechial rash and neurological, renal, and cardiac problems; this is especially the case in elderly people [16].

Israeli spotted fever caused by *R. israeli* is more severe than MSF. It is characterized by more frequent purpuric rash, thrombocytopenia, hyponatriemia and systemic complications, such as pneumonia, nephritis, encephalopathy, and occasional fatalities also in children; unlike MSF there is no «tache noire» [16]. Initially, the distribution of Israeli spotted fever rickettsia appeared to be restricted to Israel, but more recently the organism has also been isolated from patients with MSF in Portugal [17] and from *R. sanguineus* ticks collected in 1990 in western Sicily [18].

Fig. 3. Annual incidence of MSF cases (clinically ascertained) in Italy and Sicily (official reporting system, Italian Ministry of Health).

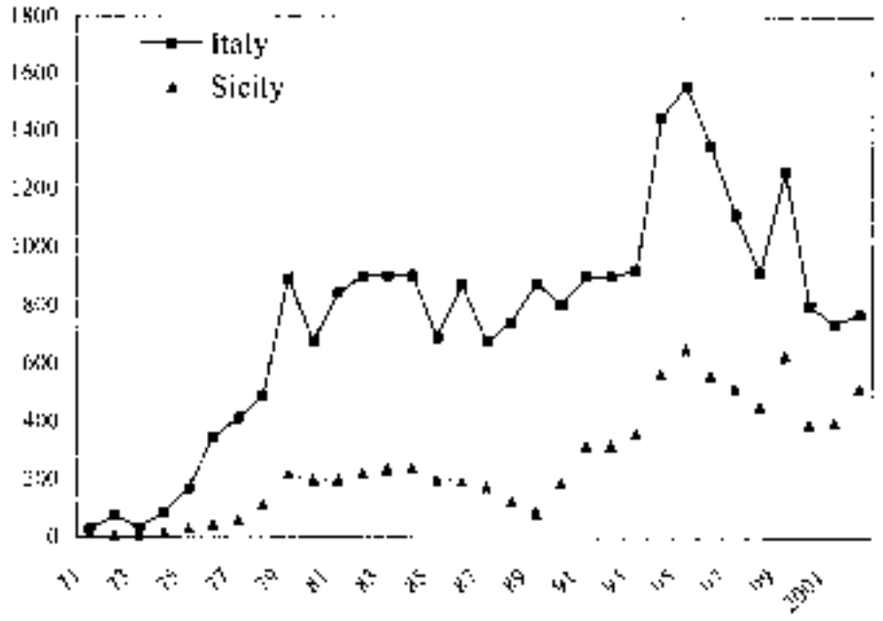
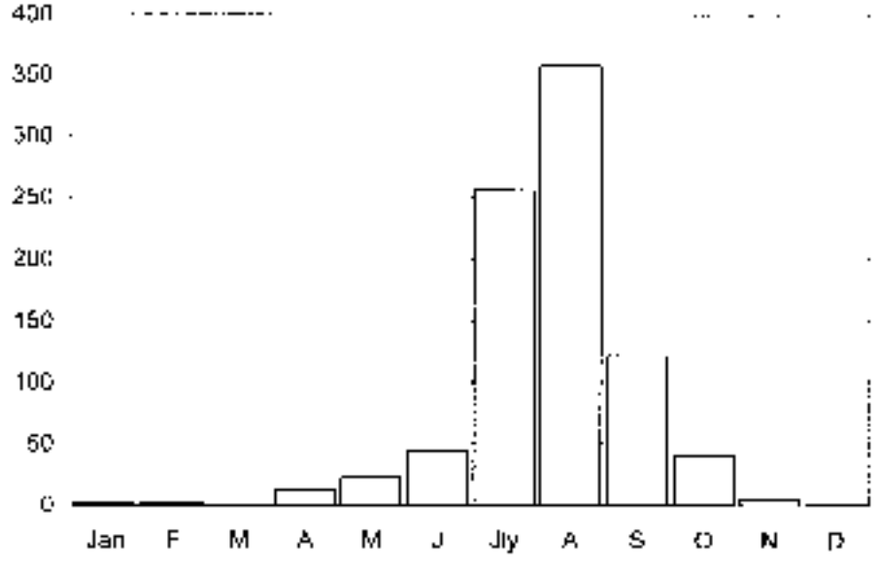


Fig. 4. Monthly distribution of 868 cases of MSF [14].



Laboratory findings

The main laboratory findings are a slight increase in transaminases and mild thrombocytopenia. Recently we have demonstrated that serum concentrations of tumor necrosis factor alpha and interferon gamma are lower in children with non-exanthematic or mild exanthematic forms as compared to children with typical exanthema [19]. These two cytokines play an important role in the defense against *R. conorii*, and are associated with the vasculitis that characterizes MSF. Milder forms could be due to a reduced activation of the immune system, resulting in a minimum albeit sufficient dose of the two cytokines to be able to destroy *R. conorii* and cause only slight tissue damage.

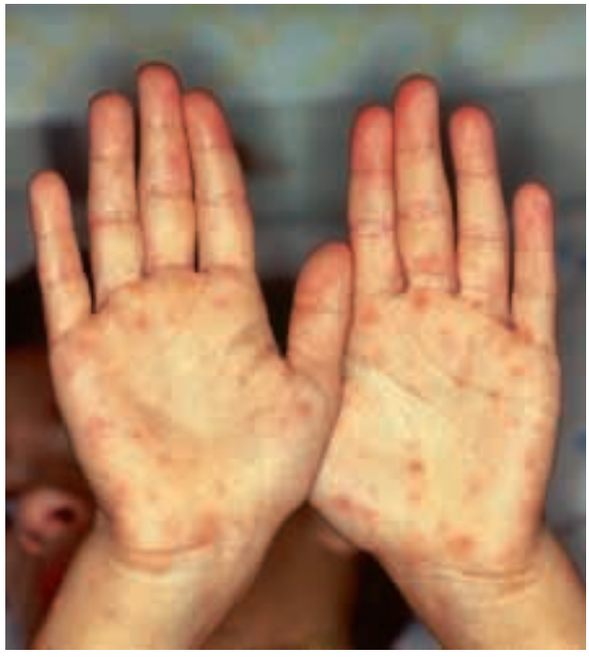
The Weil-Felix test was the first assay used to diagnose MSF and other rickettsioses. It is based on serological cross-reactions between sera from patients affected by

Tab. I. Factors alleged to explain the increase in MSF [4].

- Increased number of ticks
  - Climatic changes, i.e. «Global warming»
  - Lack of rainfalls
  - Forbidden use of D.D.T.
  - Fauna changes
    - Decreased number of tick predators
    - Increase in wild rodents and stray dogs
- Increased contact with ticks
  - Urban expansion towards rural parts of endemic areas
  - Increased leisure activities (camping)
  - Increased number of pet-dogs

rickettsial infection and somatic antigens of three *Proteus* strains: *P. vulgaris* OX2 (*R. conorii*), *P. vulgaris* OX19 (*R. rickettsii*), and *P. mirabilis* OXK (*O. tsutsugamushi*). This test lacks sensitivity and specificity. It could be used only as first line testing in rudimentary hospital laboratories. Today, the most commonly used

Fig. 5. Typical exanthema on the palms.



serological test is the IFAT [16]. However, in our experience, only titers < 1:80 of specific IgM are generally present at the onset of the disease; we have seen such low levels in other systemic diseases as well (unpublished data). Confirmation of the diagnosis of MSF thus requires documentation of a seroconversion 3-4 weeks after the onset of symptoms [1].

Recently, a shell vial system has been devised that allows detections of rickettsiae in blood within just 48 ± 72 h [20]; subsequently, an immunomagnetic procedure has been developed for the isolation of circulating endothelial cells in blood, that allows the confirmation of MSF in about 3.5 h [21]. A sensitive and specific PCR test based on sequences of the 17-kD protein gene of *R. conorii* has been developed by Leitner et al. [22]. However, these procedures are restricted to specialized laboratories.
Because of the frequent lack of several classical clinical and laboratory findings, a diagnosis score has been proposed to facilitate the diagnosis of this disease [23]. (Tab. II).

Therapy

Members of the genus *Rickettsia* are obligate intracellular bacteria, and the main feature of MSF is generalized vasculitis with localization of *R. conorii* in the cytoplasm of endothelial cells [16]. Effective intracellular concentrations of antimicrobials are therefore considered the «sine qua non» for successful treatment for this disease.
Until now there has not been a gold-standard therapy for children with rickettsial diseases. Standard treatment for MSF was the oral or parenteral administration of tetracyclines or chloramphenicol, drugs that achieve good membrane penetration and concentrate within the cytoplasm. However, both drugs can have significant adverse effects in children. Tetracyclines can cause staining of the teeth and bone toxicity [24]; chloramphenicol can cause gray-baby syndrome, aplastic anemia, and acute hemolytic anemia in patients with the Mediterranean form of glucose-6-phosphate dehydrogenase (G6PD) deficiency [25-27]. Nevertheless, doxycycline is the treatment of choice for children aged < 8 years with RMSF [28].
Until 2000 chloramphenicol was considered «the lesser of two evils», and this antibiotic was used as the drug of choice for the treatment of MSF in children [1]. The macrolides clarithromycin, azithromycin, roxithromycin, and josamycin, which are often used to treat infections in children, lack such adverse effects. The few studies that have evaluated the «in vitro» activity of these drugs against *R. conorii* [29-31] suggest that clarithromycin and azithromycin may be of interest for their better pharmacological and pharmacokinetic properties compared to other macrolides [30]. Recently, 14 hydroxy-clarithromycin, the primary metabolite of clarithromycin, has been demonstrated to attain serum concentrations 3 times greater than its MIC [29 30]. Moreover, the combination of clarithromycin and its 14-hydroxy metabolite have been shown to produce MICs and postantibiotic effects greater than those associated with the use of clarithromycin alone [29]. Since azithromycin achieves high intracellular concentrations and has a particularly long intracellular half-life, it may therefore be possible to: 1) reduce doses; 2) prolong the interval between each administration; and 3) shorten

Fig. 6. Clinical findings in 645 pediatric cases of MSF [1].

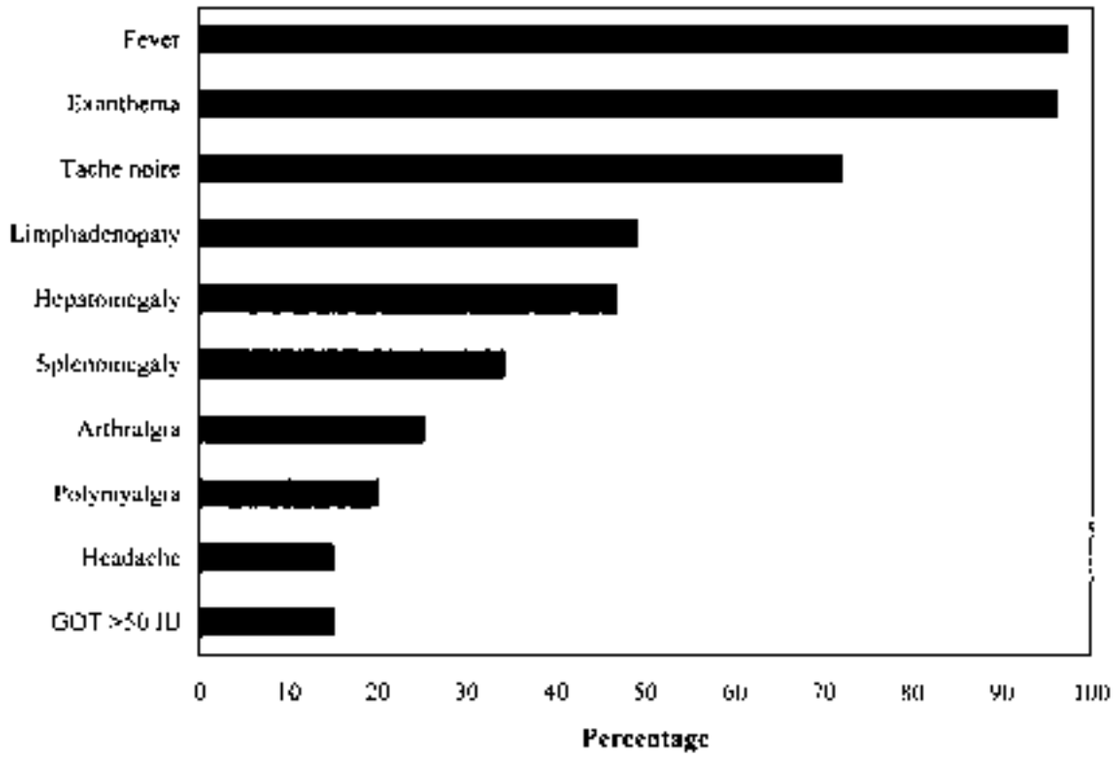
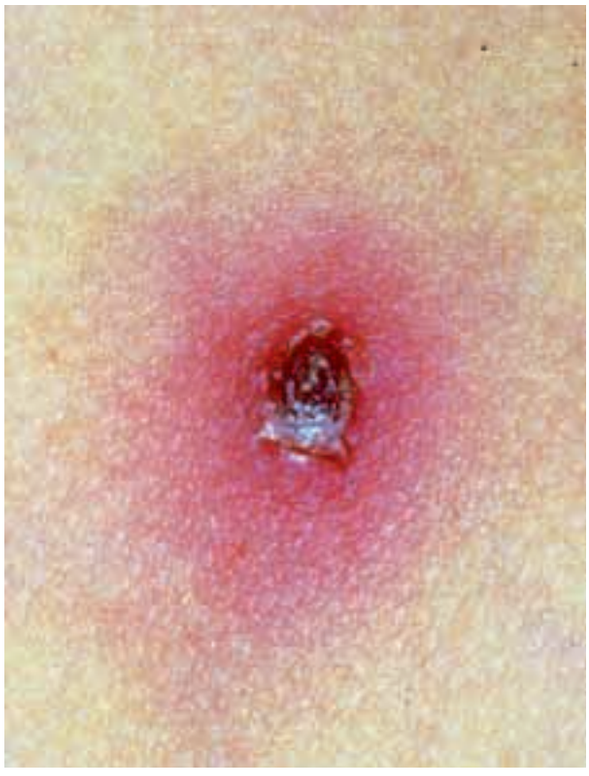


Fig. 7. Typical tache noire on the abdomen.



the duration of administration, compared to other drug treatments [32]. Given this background information, we undertook an RCT comparing the efficacy and safety of clarithromycin versus chloramphenicol to treat MSF in children, and found that clarithromycin was significantly more effective than chloramphenicol [33] (Fig. 10). Subsequently we conducted another RCT to compare the safety and efficacy of clarithromycin versus those of azithromycin. We were unable to demonstrate significant differences between the two treatment groups in terms of time to defervescence and disappearance of other symptoms [34].

Other three RCTs have been performed to compare macrolides for the treatment of MSF in children [35-37]. In all these studies, the drugs were administered orally, and the clinical response to the different antibiotic treatments was evaluated on the basis of the pattern of body temperature and the improvement of the clinical signs and symptoms of the disease. Only the study [33] that compared the efficacy of clarithromycin versus chloramphenicol obtained better (statistically significant) results for the clarithromycin group. On the other hand, only one of the other studies [36] showed that tetracycline hydrochloride is better than erythromycin stearate. However, erythromycin displayed an «in vitro» activity against *R. conorii* that was not as potent as that of newer macrolides, such as clarithromycin [29 30 38-41].

In conclusion, clarithromycin and azithromycin could be acceptable therapeutic alternatives to chloramphenicol

Fig. 8. Localization of tache noire in 645 pediatric cases of MSF [1].

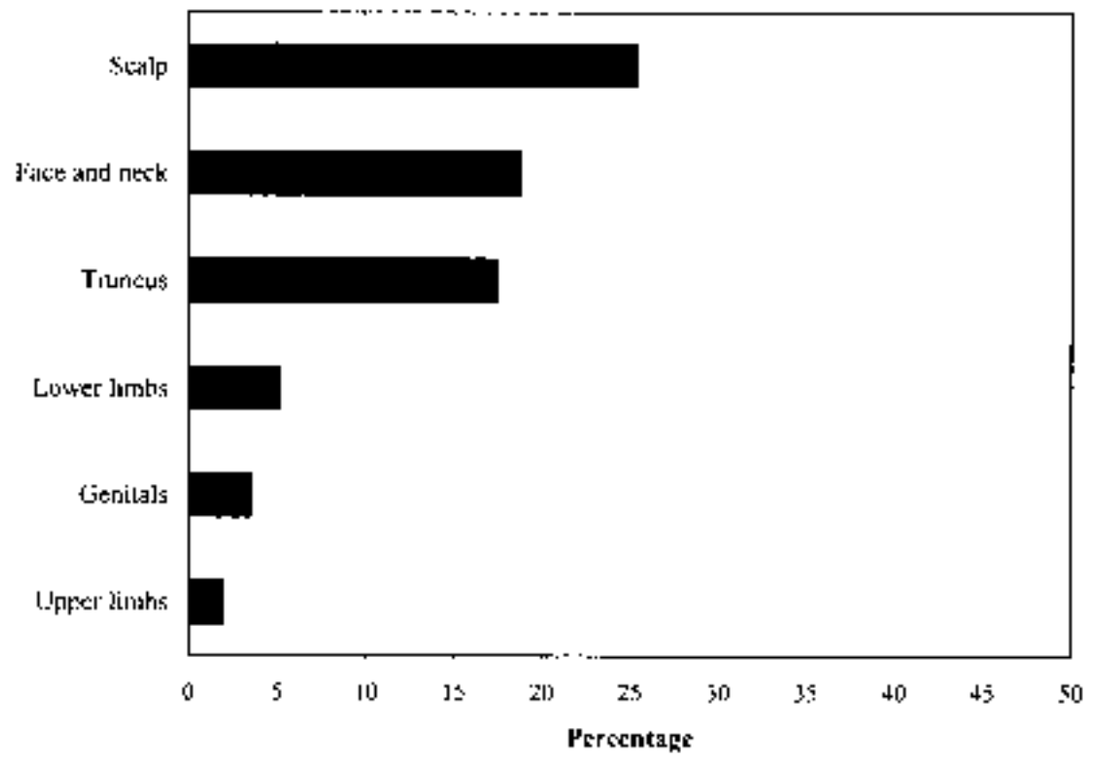
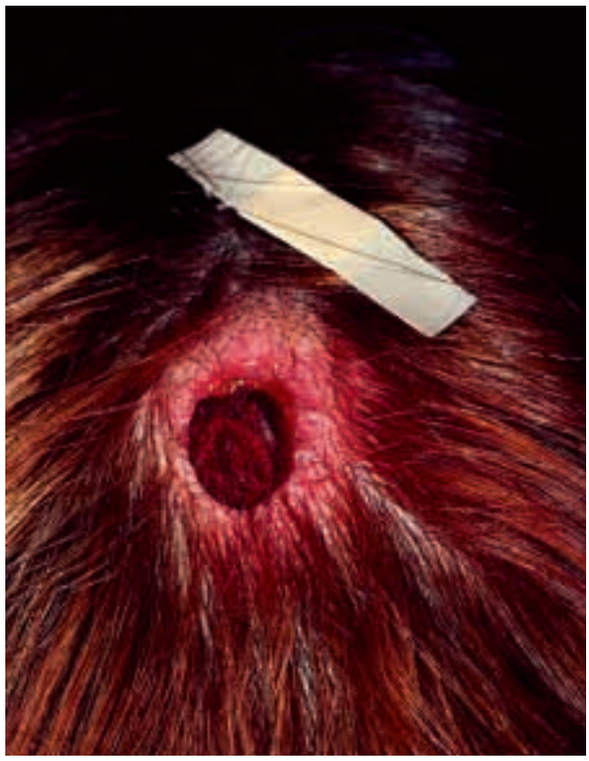


Fig. 9. Alopecia surrounding a large tache noire on the scalp.



and tetracyclines for children aged < 8 years with MSF. Since azithromycin has a long half-life, it offers the advantages of administration in a single daily dose and a shorter duration of therapy, which could increase compliance in children. Further evaluation of macrolides in patients with severe symptoms or more severe illness should be undertaken. Because these drugs have not been tested as treatment for RMSF or other rickettsioses, their use for these diseases is not recommended.

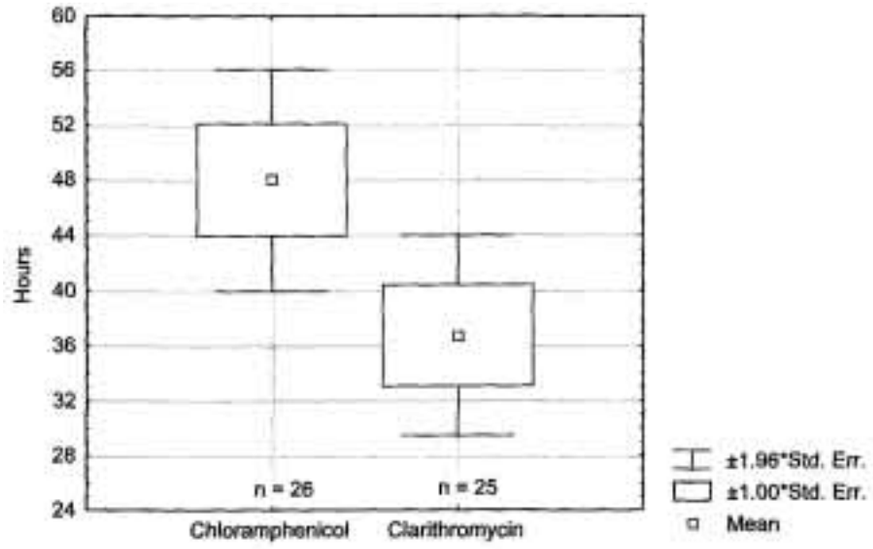
Although macrolides should not be used to treat MSF in adults until RCTs have demonstrated at least the same efficacy of chloramphenicol or tetracyclines, their use could be recommended during pregnancy.

As far as children are concerned, we think that until large studies in open populations will confirm the results of the RCTs performed [33 34], clarithromycin (7.5 mg/kg b.i.d.) and azithromycin (10 mg/kg once a day) should be administered until at least 48 hours after defervescence. In case of neurological signs, tetracyclines (minocycline or doxycycline) or chloramphenicol should be given at any age.

What to do in case of a tick bite

The tick should be removed using rounded forceps and – should immature ticks be found – with the help of a magnifying glass. The mouthparts of the ticks must be grasped as close to the skin as possible, and the tick

Fig. 10. Time to defervescence in children treated with chloramphenicol or clarithromycin [33].



Tab. II. Diagnostic scoring system for Mediterranean spotted fever, as described by Raoult et al. [23].

Diagnostic criterion	Points
Epidemiologic criteria	
Living or recent travel in endemic area	2
Onset between May and September	2
Contact with dog ticks	2
Clinical criteria	
Fever above 39 °C	5
«Tache noire»	5
Maculopapular or purpuric eruption	5
Two of the three clinical criteria	3
All three clinical criteria	5
Non specific biological criteria	
Platelet count < 150 x 10^9/liter	1
Liver enzymes (AST or ALT) > 50 IU/liter	1
Bacteriological criteria	
Detection of *R. conorii* in skin biopsy by using immunofluorescence assay	25
Isolation of *R. conorii* from blood	25
Serological criteria (immunofluorescence)	
Single serum sample with total Ig of ≥ 1:128	5
Single serum sample with IgG of ≥ 1:128 and IgM of ≥ 1:64	10
Two serum samples with fourfold titer elevation within 2 weeks	20

Note. A total of > 25 points is consistent with a presumptive diagnosis of Mediterranean spotted fever.

must then be pulled upward, perpendicular to the surface, with a steady pressure [3].

After the tick has been removed, the bite site should be disinfected thoroughly. It is not advisable to remove ticks by using the fingers instead of forceps, or try and kill them in situ with lighted cigarettes, petroleum jelly, or suntan oil: these methods, in fact, may increase the risk of regurgitation by the tick and, consequently, the transmission of infectious agents [3].

It should be kept in mind that only about 10% of *R. sanguineus* are infected by *R. conorii* in areas in which MSF is endemic; in addition, the risk of transmission of a bacterial disease by a tick increases with the duration of attachment and generally requires 24-48 h. The degree of tick engorgement or the time from tick exposure to its detection may help define the likely duration of the attachment and the risk of disease transmission. There are no data to indicate that antimicrobial pro-

Tab. III. Summary of the findings of 5 randomized clinical trials of macrolide treatment for Mediterranean spotted fever in children [34].

Reference	Drugs and regimens studied	No. of patients	Outcome
35	Erythromycin stearate, 10 days (12.5 mg/kg q.i.d) vs. tetracycline hydrochloride, 10 days (10 mg/kg q.i.d.)	81	more rapid disappearance of fever and symptoms in the group treated with tetracycline
36	Azithromycin, 3 days (10 mg/kg once a day) vs. doxycycline, 5 days (5 mg/kg once a day)	30	no statistically significant differences in time to defervescence
37	Doxycycline, 1 day (2.5 mg/kg b.i.d.) vs. josamycin, 5 days (25 mg/kg b.i.d.)	32	no statistically significant differences in time to defervescence
33	Clarithromycin, 7 days (7.5 mg/kg b.i.d.) vs. chloramphenicol, 7 days (12.5 mg/kg q.i.d.)	46	more rapid disappearance of fever in the group treated with clarithromycin
34	Azithromycin, 3 days (10 mg/kg once a day) vs. clarithromycin, 7 days (7.5 mg/kg b.i.d.)	87	no statistically significant differences in time to defervescence

phylaxis is beneficial for tick-bitten patients to prevent MSF or other rickettsioses, Lyme disease and tularemia [3]. However, patients should be alerted on the signs and symptoms of tick transmitted diseases and seek medical help in case of contact.

In the presence of a lesion ascribed to «tache noire» we recommend to start antibiotic treatment even if fever or exanthema are not present.

References

1 Cascio A, Dones P, Romano A, Titone L. *Clinical and laboratory findings of boutonneuse fever in Sicilian children.* Eur J Pediatr 1998;157:482-6.

2 Cascio G, Titone L. *Rickettsiosi*. In: Introzzi P, ed. *Enciclopedia medica italiana*. Florence: Utet Edizioni Scientifiche 1987:1364-90.

3 Parola P, Raoult D. *Ticks and tickborne bacterial diseases in humans: an emerging infectious threat.* Clin Infect Dis 2001;32:897-928.

4 Cascio A, Scarlata F, Giordano S, Bosco M, Cascio G. *Mediterranean spotted fever in Sicily. Epidemiological and clinical considerations with regard to a pediatric series.* Mediterr J Infect Parasitic Dis 1994;9:75-81.

5 Randolph SE. *Ticks are not insects: consequences of contrasting vector biology for transmission potential.* Parasitology Today 1998;14:186-91.

6 Santos AS, Bacellar F, Santos-Silva M, Formosinho P, Gracio AJ, Franca S. *Ultrastructural study of the infection process of Rickettsia conorii in the salivary glands of the vector tick Rhipicephalus sanguineus*. Vector Borne Zoonotic Dis 2002;2:165-77.

7 Shaw SE, Day MJ, Birtles RJ, Breitschwerdt EB. *Tick-borne infectious diseases of dogs*. Trends in Parasitology 2001;17:74-80.

8 Mumcuoglu KY, Frish K, Sarov B, Manor E, Gross E, Gat Z, Galun R. *Ecological studies on the brown dog tick Rhipicephalus sanguineus (Acari, Ixodidae) in southern Israel and its relationship to spotted-fever group rickettsiae*. J Med Entomol 1993;30:114-21.

9 Mansueto S, Tringali G, Walker DH. *Widespread simultaneous increase in the incidence of spotted fever group rickettsioses.* J Infect Dis 1986;154:539-40.

10 Walker DH, Fishbein DB. *Epidemiology of rickettsial diseases.* Eur J Epidemiol 1991;7:237-45.

11 Staszewski S, Helm FB, Stille W. *Autochthonic Mediterranean spotted fever in West Germany*. Lancet 1984;II:1466.

12 McDonald JC, MacLean JD, McDade JE. *Imported rickettsial disease: clinical and epidemiological features*. Am J Med 1988;85:799-805.

13 Gilot B, Laforge ML, Pichot J, Raoult D. *Relationships between the Rhipicephalus sanguineus complex ecology and Mediterranean spotted fever epidemiology in France*. Eur J Epidemiol 1990;6:357-62.

14 Cascio A, Colomba C. *Macrolides in the treatment of children with Mediterranean spotted fever*. Infez Med 2002;10:145-50.

15 Radulovic S, Feng H, Morovic M, Crocquet-Valdes P, Walker DH. *Isolation of Rickettsia akari from a patient in a region where Mediterranean spotted fever is endemic.* Clin Infect Dis 1995;22:216-20.

16 Raoult D, Roux V. *Rickettsioses as paradigms of new or emerging infectious diseases.* Clin Microbiol Rev 1997;10:694-719.

17 Bacellar F, Beati L, Franca A, Pocas J, Regnery R, Filipe A. *Israeli spotted fever rickettsia (Rickettsia conorii complex) associated with human disease.* Emerg Infec Dis 1999;5:835-6.

18 Giammanco GM, Mansueto S, Ammatuna P, Vitale G. *Israeli Spotted Fever Rickettsia in Sicilian Rhipicephalus sanguineus Ticks.* Emerg Infec Dis 2003;9:892-3.

19 Cascio A, Gervasi F, Giordano S, Palazzolo B, Salsa L. *Plasma levels of tumor necrosis factor α and interferon γ in Sicilian children with Mediterranean spotted fever*. Int J Clin Lab Res 1997;27:135-8.

20 Marrero M, Raoult D. *Centrifugation-shell vial technique for rapid detection of Mediterranean spotted fever Rickettsia in*

blood culture. Am J Trop Med Hyg 1989;40:197-9.

[21] Drancourt M, George F, Brouqui F, Sampol J, Raoult D. *Diagnosis of Mediterranean spotted fever by indirect immuno fluorescence of Rickettsia conorii in circulating endothelial cells isolated with monoclonal antibody-coated immunomagnetic beads*. J Infect Dis 1992;166:660-3.

[22] Leitner M, Yitzhaki S, Rzotkiewicz S, Keysary A. *Polymerase chain reaction-based diagnosis of Mediterranean spotted fever in serum and tissue samples.* Am J Trop Med Hyg 2002;67:166-9.

[23] Raoult D, Tissot-Dupont H, Chicheportiche C, Peter O, Gilot B, Drancourt M. *Mediterranean spotted fever in Marseille, France: correlation between prevalence of hospitalized patients, seroepidemiology, and prevalence of infected ticks in three different areas.* Am J Trop Med Hyg 1993;48:249-56.

[24] American Academy of Pediatrics Committee on Drugs. *Requiem for tetracycline*. Pediatrics 1975;55:142-3.

[25] Luzzatto L. *G6PD deficiency and haemolytic anemia*. In: Nathan G, Oski FA, eds. *Hematology of infancy and childhood. 4th Ed*. Philadelphia: Saunders, 1993:674-731.

[26] Craft AW, Brocklebank JT, Hey EN, Jackson RH. *The «gray toddler»: chloramphenicol toxicity.* Arch Dis Child 1974; 49:235-7.

[27] Wallerstein RO, Condit PK, Kasper CK, Brown JW, Morrison FR. *Statewide study of chloramphenicol therapy and fatal aplastic anemia*. JAMA 1969;208:2045-50.

[28] American Academy of Pediatrics. *Rocky Mountain spotted fever*. In: Peter G, ed. *Red Book. Report of the committee on infectious diseases. 26th Ed.* Elk Grove Village, IL: Am Acad Pediatr 2003:452-4.

[29] Ives TJ, Marston EL, Regnery RL, Butts JD, Majerus TC. *In vitro susceptibilities of Rickettsia and Bartonella spp. to 14- hydroxy-clarithromycin as determined by immunofluorescent antibody analysis of infected Vero cell monolayers*. J Antimicrob Chemother 2000;45:305-10.

[30] Ives TJ, Manzewitsch P, Regnery RL, Butts JD, Kebede M. *In vitro susceptibilities of Bartonella henselae, B. quintana, B. elizabethae, Rickettsia rickettsii, R. conorii, R. akari, and R. prowazekii to macrolide antibiotics as determined by immunofluorescent-antibody analysis of infected Vero cell monolayers.* Antimicrob Agents Chemother 1997;41:578-82.

[31] Rolain JM, Maurin M, Vestris G, Raoult D. *In vitro susceptibilities of 27 Rickettsiae to 13 antimicrobials*. Antimicrob Agents Chemother 1998;42:1537-41.

[32] Wildfeur A, Laufen H, Zimmermann T. *Uptake of azithromycin by various cells and its intracellular activity under in vivo conditions.* Antimicrob Agents Chemother 1996;40:75-9.

[33] Cascio A, Colomba C, Di Rosa D, Salsa L, di Martino L, Titone L. *Efficacy and safety of clarithromycin in the treatment of Mediterranean spotted fever in children: a randomized controlled trial.* Clin Infect Dis 2001;33:409-11.

[34] Cascio A, Colomba C, Antinori S, Paterson DL, Titone L. *Clarithromycin versus azithromycin in the treatment of Mediterranean spotted fever in children: a randomized controlled trial.* Clin Infect Dis. 2002;34:154-8.

[35] Munoz-Espin T, Lopez-Pares P, Espejo-Arenas E, Font-Creus B, Martinez-Vila I, Traveria-Casanova J, et al. *Erythromycin versus tetracycline for treatment of Mediterranean spotted fever.* Arch Dis Child 1986;61:1027-9.

[36] Meloni G, Meloni T. *Azithromycin vs doxycycline for Mediterranean spotted fever.* Pediatr Infect Dis J 1996;15:1042-4.

[37] Bella F, Font B, Uriz S, Munoz T, Espejo E, Traveria J, et al. *Randomized trial of doxycycline versus josamycin for Mediterranean spotted fever.* Antimicrob Agents Chemother 1990;34:937-8.

[38] Maurin M, Raoult D. *In vitro susceptibilities of spotted fever group Rickettsiae and Coxiella burnetii to clarithromycin*. Antimicrob Agents Chemother 1993;37:2633-7.

[39] Drancourt M, Raoult D. *In vitro susceptibilities of Rickettsia rickettsii and Rickettsia conorii to roxithromycin and pristinamycin*. Antimicrob Agents Chemother 1989;33:2146-8.

[40] Raoult D, Rousselier P, Tamalet J. *In vitro evaluation of josamycin, spiramycin, and erythromycin against Rickettsia rickettsi and R. conorii.* Antimicrob Agents Chemother 1988;32:255-6.

[41] Keysary A, Itzhaki A, Rubinstein E, Orou C, Keren G. *The invitro antirickettsial activity of macrolides.* J Antimicrob Chemother 1996;38:727-31.