

Continuous alert for rickettsiosis in Sicily: molecular characterization of rickettsia sp. obtained from ticks and human beings (1986-2001)

Giovanni Maurizio Giammanco¹, Salvatore Di Rosa², Pietro Ammatuna¹, Pasquale Mansueto³, Anna Micalizzi³, Giustina Vitale³

¹Dipartimento di Igiene e Microbiologia, Università di Palermo, Italy;

²Ospedale Villa Sofia Whitaker, Palermo, Italy;

³Dipartimento di Medicina Clinica e delle Patologie emergenti, Università di Palermo, Italy

SUMMARY

Several strains of Rickettsia sp. were isolated from patients in western Sicily with MSF (Mediterranean Spotted Fever) as well as ticks. Strains isolated were examined by PCR and identified as belonging to *R. conorii* sp. Importantly a strain of Israeli Spotted Fever Rickettsiae, obtained from a tick, was also identified. Our data prove that strains other than the classical *R. conorii* also circulate in Sicily.

KEY WORDS: Ticks, Rickettsiae.

Received June 29, 2005

Accepted August 2, 2005

The recent sharp increase in the incidence of Mediterranean Spotted Fever (MSF) also defined as Boutonneuse fever in the western hemisphere, especially in France, Spain and Italy, prompted us to investigate the clinical, epidemiological and microbiological aspects of the disease (Mansueto *et al.*, 1986; Mansueto, 1997).

In the course of a specific plan of work, we isolated for the first time several strains of Rickettsia sp. from Sicilian patients with MSF as well as from ticks in western Sicily.

1515 ticks collected at different sites of our region were examined by hemolymph test (Burgdorfer, 1970). Hemolymph was collected by amputating

the distal tarsal segment of one or more legs, absorbed on a glass slide and examined by indirect immunofluorescence test with a high-titred human anti-*R. conorii* serum to which anti-human immunoglobulin labelled with fluorescein isothiocyanate was later added. Ticks were considered positive when clearly fluorescent on microscopic examination. Positive ticks were triturated, washed in succession with several solutions (water, ethanol and antibiotics) and used to infect VERO cells for isolation of strains according to the method described in detail elsewhere (Tringali *et al.*, 1986; Vitale *et al.*, 1989). Human strains were obtained from the blood of 180 patients, who gave informed consent, with symptoms and signs that led us to suspect Boutonneuse fever (fever, maculopapular exanthema, eschar at the site of the tick bite and specific antibody titer at significant level - $\geq 1/80$ - at recovery or at seven days interval). Heparinized blood was collected and inoculated into VERO cells (Mansueto *et al.*, 1995).

Corresponding author

Giustina Vitale
c/o Dipartimento di Medicina Clinica
e delle Patologie Emergenti
Università di Palermo
Via del Vespro, 141
90127 Palermo
e-mail: vitale@unipa.it

Strains isolated from *Rhipicephalus sanguineus* and from patients were identified by molecular-sequence-based identification techniques. Bacterial DNA was examined by polymerase chain reaction (PCR) for *ompA* gene and restriction analysis under conditions previously described by Roux *et al.* (Giammanco *et al.*, 2003; Roux *et al.*, 1996). Bacterial DNA was obtained from 200 µl of infected Vero cell suspensions and the PCR was performed with primer pair Rr 190.70p and 190.701, which amplifies 632bp portions of the *ompA* gene. Amplified products were submitted to restriction analysis with endonucleases *PstI* and *RsaI* (Amersham Pharmacia Biotech) and restriction fragments were resolved on 10% polyacrylamide gels.

Of 1515 ticks, 312 (20.6%) were positive for the hemolymph test. At the same time we also isolated and cultivated 27 strains of rickettsia sp. from patients and 20 strains of organism from ticks (Table 1). So far, acarine and human strains in the majority of cases seem to be members of *R. conorii* sp. but what is important is that we also identified a strain from tick belonging to the Israeli Spotted Fever Rickettsiae.

The premise of the present work was the clinical observation that the disease is mild in most cases but on some occasions may be more severe. Hypothetically these differences may be due to the different virulence of strains and/or to the circulation of strains other than classical Rickettsiae sp. as well as, of course, to different manifestations of the disease in patients (Vitale *et al.*, 1989). Our data prove that strains other than the classical *R. conorii* also circulate in Sicily (e.g. Israeli Spotted Fever Rickettsiae).

Since a similar organism was isolated from patients with MSF in Portugal (Bacellar *et al.*, 1999), the same circumstance cannot be excluded in our setting. These data are in accordance with the results obtained in several other countries. So, *R. slovaca*, *R. mongolotimonae* and *R.*

conorii circulate in southern France; *R. africana* and *R. conorii* in southern Africa and in Portugal (Bacellar *et al.*, 1999).

The purpose of future work will be:

- 1) to identify the strains collected so far to define the Sicilian assessment of rickettsiae and
- 2) more importantly, to correlate the severity of clinical cases with the microbiological characteristics of cultivated organisms.

In the light of the reported data, we can conclude that in Sicily Boutonneuse fever is certainly caused by *R. conorii* but also by other species, not identified to date. Further investigation is necessary to verify if -as it seems- these "different" strains give rise to more serious forms of Boutonneuse fever. This study is in progress at our institution.

ACKNOWLEDGEMENTS

We are grateful to Dr. Sheila McIntyre for her help in the preparation of the manuscript.

REFERENCES

- BACELLAR, F., BEATI, L., FRANCA, A., POCAS, J., REGNER, R., FILIPE, A. (1999). Israeli spotted fever rickettsia (*Rickettsia conorii* complex) associated with human disease. *Emerging Infectious Diseases*, **5**, 835-836.
- BURGDORFER W. (1970). Hemolymph test: a technique for detection of rickettsiae in ticks. *Am J Trop Med Hyg*, **19**, 1010-14.
- GIAMMANCO, G.M., MANSUETO, S., AMMATUNA, P., VITALE, G. (2003). Israeli Spotted Fever Rickettsia in Sicilian *Rhipicephalus sanguineus* Ticks. *Emerging Infectious Diseases*, **9**, 892-893.
- MANSUETO, S. (1997). "Le Rickettsiosi". 98° Proceedings of the Italian Society of Internal Medicine. Il Policlinico, **104-9/10**, Ed. Luigi Pozzi-Roma.
- MANSUETO, S., TRINGALI, G., WALKER, D.H. (1986). Widespread, simultaneous increase in the incidence of spotted fever rickettsioses. *Journal of Infectious Diseases*, **154**, 539-540.
- MANSUETO, S., ROTONDO, G., GAMBINO, G., MOCCIARO, C., SPINELLI, A., FARINELLA, E., OCCHINO, C., DI GRIGOLI, C., D'ANCONA, F., VITALE, G. (1995). Attempts to isolate spotted fever rickettsiae from patients with clinically diagnosed boutonneuse fever. *Mediterranean Journal of Infectious and parasitic diseases*, **10**, 127-131.
- ROUX, V., FOURNIER, P.E., RAOULT, D. (1996). Differentiation of spotted fever group *Rickettsiae*

TABLE 1 - *Rickettsia* spp from Sicilian ticks and humans.

	Examined	Isolated strains (%)
Ticks	1515	20 (6.4%)
Humans	180	27 (30%)

- by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein *rOmpA*. *Journal of Clinical Microbiology*, **34**, 2058-65.
- TRINGALI, G., INTONAZZO, V., PERNA, AM, MANSUETO, S., VITALE, G., WALKER, DH. (1986). Epidemiology of Boutonneuse fever in western Sicily. Distribution and prevalence of spotted fever group rickettsial infection in dog ticks (*Rhipicephalus sanguineus*). *American Journal of Epidemiology*, **123**, 721-727.
- VITALE, G., DI STEFANO, R., DAMIANI, G., MANSUETO, S. (1989). Characterization of Sicilian strains of spotted fever group rickettsiae by using monoclonal antibodies. *Journal of Clinical Microbiology*, **27**, 1081-1085.