Detection and characterization of Rickettsial strains in ticks from Sardinia, Italy

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Ticks are of considerable medical and veterinary importance, because they harm the host through their feeding action and so transmit a lot of pathogens. Tick-trasmitted pathogens are quite diverse and include organisms belonging to the genera *Rickettsia, Ehrlichia, Anaplasma, Borrelia, Francisella, Cowdria* and *Coxiella.* In Sardinia, the cases of tick bite related fever are considerably higher than in the rest of Italy: an average rate of 11.9 cases year for every 100,000 inhabitants versus a national average of 2.1 cases year for 100,000 inhabitants has been reported (Beninati T *et al,* 2002, Emerging Infectious Diseases, 9: 983-986).
The aim of this study was, firstly, to detect the presence of Rickettsial DNA by PCR and, then, to identify the Rickettsiae species using restriction endonuclease fragment length polymorphism (RFLP) on two amplified genes.
Tick collection: Between December 2004 to December 2005 a total of 1770 adult ticks was collected from different animals (muflon, deer, hedgehog, sheep, goat, horse, cattle, dog , cat, etc.) and vegetation in two provinces (Ogliastra and Cagliari). Ticks were stored in vials containing 70% ethanol and identified using basic taxonomic schemes.
DNA extraction and PCR amplification: ticks from each animal were before pooled according to their species and sex and after homogenized in STE buffer. DNA was extracted and purified following the procedure described by Wu-Chun Cao *et al* (2000, Journal of Clinical Microbiology, 38: 2778-2780).
PCR amplification was performed by using oligonucleotide primers derived from the 120 kDa outer membrane protein (ompB) gene of *Rickettsia rickettsii* as described by Noda H *et al* (1997, Applied Environ Microbiol, 63: 3929-3932).
PCR amplification of DNA was verified by electrophoresis in 1% agarose gel. Positive samples were subjected to two further amplifications using primer sets from ompA and citrate syntase (gltA) genes respectively (Regnery R *et al,* 1991, J of Bact, 173: 1576-1589).
Amplicon from ompA (536 bp) was digested with *Pst*I and *Rsa*I endonucleases whereas amplicon from gltA (353 bp) was cleaved with *Alu*I restriction enzyme at 37°C for 16 hours. The digested reactions were loaded on 1 mm thick, 12% polyacrylamide vertical gels. Gels were run at 100 V for 4 hours, stained with ethidium bromide and examined by UV lamp in an ImageMaster VDS-CL (Amersham). DNA molecular mass marker VIII (fragment mixture prepared by cleavage of pUCBM21 DNA with restriction endonucleases Hpa II and Dra I plus Hind III - Roche) was run with the samples to determine the molecular masses of the DNA fragments.
Results: Preliminary electrophoretic analysis of uncut PCR products suggested that all rickettsial ompB PCR amplified products were similar. Of a total of 210 DNA samples 14 (6.7%) were PCR-positive for ompB gene. Six of these samples were subjected to RFLP analysis. The results have permitted to identify only one strain as *R. africae.* Further analyses are in progress to study the other species.