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Letter to the Editor

Brucellosis in a dog caused by *Brucella melitensis* Rev 1

Brucella melitensis was isolated from a dog that suffered from recurrent fever, discospondylitis and dysuria before it died in spite of antibiotic therapy. The strain was analyzed extensively by phenotypic and genetic methods and found to be identical to vaccine strain *B. melitensis* Rev 1.

Canine brucellosis is caused by *Brucella canis* and characterized mostly by orchitis and epididymitis in males, abortion in females, poor reproductive performance in both sexes, lymphadenopathy and discospondylitis. In some cases the animal may be asymptomatic. Since *B. canis* lacks lipopolysaccharides that are present in the smooth species of *Brucella*, fever is an uncommon sign in typical canine brucellosis (Hollett, 2006). The animal from this case was a 5-year-old male (not neutered) Dachshund weighing 12 kg, living in the area of Athens, Greece. The animal had recurrent fever of 40–41 °C over a period of 3 months, and upon presentation to the veterinary clinic showed mild anorexia, spondyloarthritis and dysuria. The dog had no obvious contact to farm animals. It was fed mainly with homemade food which was supplemented with vitamins and minerals during the hunting period. The treatment with 6.25 mg/lb amoxicillin-clavulanic acid (CLAVAMOX, Pfizer Animal Health) plus corticosteroids 0.5 mg/kg (DELTACORTRIL 5 mg tablets, Pfizer) twice a day per os over a period of 15 days was not successful. The treatment was continued with vibramycin 5 mg/kg (VIBRAMYCIN SIROP 50 MG/5 ML, Petline) plus corticosteroids 0.5 mg/kg, followed by lincomycin hydrochloride and spectinomycin sulfate tetrahydrate 5 mg/kg (LINCOSPECTIN, Pharmacia & Upjohn) administered intramuscularly and corticosteroids 0.5 mg/kg for 10 days, again without success. Serum levels of urea (58 mg/dl, ref. range 6–25 mg/dl) and liver enzymes (ALT 135 iu/l, ref. range 12–18 iu/l; AP 178 iu/l, ref. range 5–131 iu/l, and AST 212 iu/l, ref. range 15–66 iu/l) were significantly increased. One month after the last antibiotherapy the dog died after suffering from high pyrexia, anorexia and diarrhea. The necropsy showed an enlargement of the liver. Serological tests for *Leishmania donovani* and *Leptospira* spp. were negative, while the Rose-Bengal test used for diagnosis of infection with smooth *Brucella* species was positive. Prostatic fluid cultured on *Brucella*-selective agar for 72 h at 37 °C resulted in small grey colonies that were identified as *Brucella* spp. by standard phenotypic identification. Species-specific PCR analysis (Hinic et al., 2008) of this *Brucella* isolate, revealed *Brucella melitensis*. The isolate was stored in our strain collection as strain JF4519. Knowing that *B. melitensis* vaccine strain Rev 1 is used extensively in Greece for vaccination of small ruminants, strain JF4519 was analyzed in detail by phenotypic and genetic methods and compared to *B. melitensis* vaccine strain Rev 1 (batch A11/92, S.S. Elberg, purchased from EDQM, Council of Europe, Strasbourg, France) and to *B. melitensis* type strain 16 M (ATCC 23456). The minimal inhibitory concentrations (MIC) for type strain 16 M was >32 μg/ml for benzylpenicillin and 0.75 μg/ml for streptomycin. In contrast, both strain JF4519 isolated from the dog and vaccine strain Rev 1 had MICs of 0.032 μg/ml for benzylpenicillin and of 2.5 μg/ml for streptomycin, indicating that JF4519 could be identical to strain Rev 1. Subsequent PCR-*Nco*I RFLP analysis (Cloeckaert et al., 2002) of strain JF4519 and strain Rev 1 resulted in a single fragment of 510 bp, lacking the *Nco*I restriction site for both. The *Nco*I restriction site in this PCR amplification is characteristic for type strain 16 M that showed two fragments of 348 bp and 162 bp. Sequence analysis of the *rps*L (ribosomal small subunit protein L) gene that was amplified by PCR revealed for both *B. melitensis* strain JF4519 and Rev 1 the characteristic mutation CTG (instead of CCG) at codon position 91 giving rise to the amino acid change $Pro_{91} \rightarrow Leu$ responsible for the streptomycin resistance found in JF4519 and Rev 1. In contrast, *B. melitensis* type strain 16 M revealed the wild type sequence CCG at codon position 91 of the *rps*L gene. In order to further confirm the identity or close similarity between strain JF4519 isolated from the dog and vaccine strain Rev 1, we have performed multiple locus variable number tandem repeat analysis (MLVA) of these two strains plus type strain 16 M as control, using the primers described elsewhere (García-Yoldi et al., 2007) and the Agilent 2100 Bioanalyzer platform for DNA sizing (Agilent Technologies, Santa Clara, CA, USA). MLVA analysis using all 15 markers resulted in an identical profile between JF4519 and Rev 1 corresponding to "genotype 4" which is typical for most of Rev 1 isolates, including the culture collection Elberg Rev 1 reference strain (García-Yoldi et al., 2007). The strain 16 M showed the profile corresponding to "genotype 1" described by García-Yoldi et al. (2007), differing in the microsatellite loci Bruce 07, Bruce 09 and Bruce 18 from the strains JF4519 and Rev 1 (Fig. 1). The data obtained with this most discriminatory method currently available for subtyping strains of *B. melitensis* strongly indicate that

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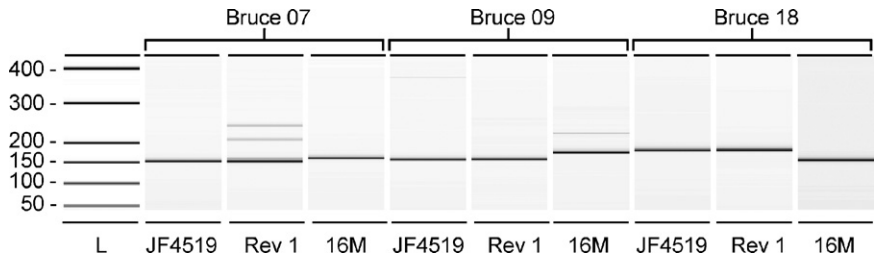

Fig. 1. Amplification products of the microsatellite loci Bruce 07, Bruce 09 and Bruce 18 of *B. melitensis* strains JF4519, Rev 1 and 16 M analyzed on the Agilent 2100 Bioanalyzer (electrophoresis image). L = DNA Ladder.

the *B. melitensis* isolate from the dog (strain JF4519) is identical to vaccine strain Rev 1. *B. melitensis* strain Rev 1 is a live attenuated vaccine strain that is used for the control of brucellosis in small ruminants in many countries including Greece (Minas et al., 2004). Although the reversion of Rev 1 to a virulent phenotype is unlikely (Ne'eman, 1968a,b), Rev 1 is able to induce abortions in pregnant animals and was also found excreted in milk of adult vaccinated animals (Banai, 2002). Virulence of Rev 1 strain for humans is well documented (Blasco and Diaz, 1993; Banai, 2002; Grilló et al., 2006). Although the owners of the dog had no particular recollection of a contact of their dog with farm animals, it is conceivable that the animal got infected directly by contact or indirectly via contaminated aborted fetus or milk of vaccinated animals. Our finding is the first report of isolation of *B. melitensis* with pheno- and genotypes identical to vaccine strain Rev 1 from a diseased dog with pyretic brucellosis. This report merits attention in the view of possible transmission of the infection from companion animals to humans.

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Vladimira Hinić
Isabelle Brodard
Institute of Veterinary Bacteriology, National Centre for Zoonoses, Bacterial Animal Diseases and Antimicrobial Resistance (ZOBA), Vetsuisse Faculty, University of Bern, Bern, Switzerland

Evanthia Petridou
George Filioussis
Department of Microbiology and Infectious Diseases, Aristotle University of Thessaloniki, Thessaloniki, Macedonia, Greece

Vasilis Contos
Department of Veterinary Public Health, National School of Public Health, Athens, Greece

Joachim Frey*
Carlos Abril
Institute of Veterinary Bacteriology, National Centre for Zoonoses, Bacterial Animal Diseases and Antimicrobial Resistance (ZOBA), Vetsuisse Faculty, University of Bern, Bern, Switzerland

*Corresponding author at: Institute of Veterinary Bacteriology, University of Bern, Vetsuisse Faculty, Länggass-Strasse 122, P.O. Box, CH-3001 Bern, Switzerland. Tel.: +41 0 31 631 24 14; fax: +41 0 31 631 26 34
E-mail address: joachim.frey@vbi.unibe.ch (J. Frey)

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