Characteristics of *Brucella* strains isolated from animals in Poland

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Abstract

A total of 42 *Brucella* strains were isolated from animals in Poland in years 2003-2012. Most of them (N=37) originated from wild animals, 3 from cattle, 1 from pig and 1 from sheep. The strains were characterised using both bacteriological and molecular (Bruce-ladder and MLVA) methods. The examinations revealed that all strains from wild boars, hares, cattle and pigs (N=41) had the same phenotypic characteristics and were classified as *B. suis* biovar 2. The remaining strain, isolated from sheep, was classified as *B. ovis*. The molecular examination showed that all *B. suis* biovar 2 strains, except one, had the same molecular profile as reference strain *B. suis* bv2 Thomsen. Different from the others strain originated from boars imported to Poland and its VNTR profile was typical for Iberian strains.

Key words: brucellosis, animals, *Brucella* strains, profiles

Introduction

Brucellosis is an infectious disease, affecting many species of animals and man, caused by bacteria of the genus *Brucella*. The genus encompasses ten species: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, *B. cetaceae*, *B. pinnipediae*, *B. microti* and *B. inopinata*. The main role in animals play: *B. abortus*, responsible for bovine brucellosis, *B. melitensis*, the main agent of ovine and caprine brucellosis, and *B. suis*, which causes brucellosis in pigs. The testing is based almost entirely on serological assays. But unequivocal diagnosis of *Brucella* infection can be made only by the isolation and identification of the agent. The aim of the study was to analyze and characterize all *Brucella* strains isolated in years 2003-2012 in NRL for Brucellosis of the National Veterinary Research Institute in Pulawy. The Laboratory examines all *Brucella* strains isolated from animals in Poland.

Materials and Methods

Bacterial strains. A total of 42 *Brucella* strains were isolated from animals in years 2003-2012. In this number of strains 3 originated from cattle (isolates from 5 animals), 1 from pigs (isolates from 7 boars imported to Poland), 1 from sheep (isolates from 6 rams), 25 from wild boars and 12 from hares.

Bacteriological examination. A Farrell's medium and serum dextrose agar were used for culture of specimens. The plates were incubated for 10 days at 37°C in an atmosphere with 5-10% CO₂ added. As regards material from cows, in parallel, the specimens were cul-
tured in similar conditions on a liquid medium for up to 6 weeks with weekly subcultures onto a solid selective medium. Colonies typical for Brucella were first checked with a polyclonal anti-Brucella serum, then examined in catalase and oxidase tests and stained by Gram’s method. Further characteristics were followed by using monospecific anti-A, anti-M and anti-R sera as well as tests for CO₂ requirement, production of H₂S, urease activity, growth in the presence of thionin and basic fuchsin, and lysis by phages (Tbilisi at its fast rate) tests, no CO₂ requirement for growth, no H₂S positive results in oxidase, catalase and urease (very same characteristics: agglutination with a polyclonal characteristics. Fleche et al. 2006), were applied to confirm fenotypic characteristics. (VNTR), which allows typing for a biovar level (Le Fleche P, Jacques I, Grayon M, Al Dahouk S, Bouchon P, Denouëf F, Nockler K, Neubauer H, Guilloteau LA, Vergnaud G (2006) Evaluation and selection of tandem repeat loci for Brucella MLVA typing assay. BMC Microbiol 6: 9. Lopez-Goni I, Garcia-Yoldi D, Martin CM, de Miguel MJ, Munoz PM, Blasco JM, Jacques I, Grayon M, Cloeckerta A, Ferreira AC, Cardoso R, Correa de Sa MI, Walravens K, Albert D, Garin-Bastuji B (2008) Evaluation of a multiplex PCR assay (Bruce-ladder) for molecular typing of all Brucella strains to a species level (Lopez-Goni et al.2008) and a multi-locus analysis (MLVA – panel 1) of variable number tandem repeats (VNTR), which allows typing for a biovar level (Le Fleche et al. 2006), were applied to confirm fenotypic characteristics.

**Results and Discussion**

The bacteriological examinations revealed that all strains from cows, pigs, wild boars and hares had the same characteristics: agglutination with a polyclonal anti-Brucella serum and monospecific anti-A serum, positive results in oxidase, catalase and urease (very fast rate) tests, no CO₂ requirement for growth, no H₂S production, growth on thionin, no growth on basic fuchsin, and lysis by TB phages at a concentration 10⁴ × RTD. These characteristics are typical for B. suis bv. 2 (Alton et al. 1988). On the other hand, the strain originated from sheep gave negative results in oxidase and urease tests, agglutinated only with a monospecific anti R serum and was susceptible to Brucella R/C phage, what is typical for rough strains of Brucella. The Bruce-ladder PCR assay confirmed that all examined Brucella isolated from cows, pigs, wild boars and hares are B. suis. Seven DNA fragments were amplified: 1,682, 1,071, 794, 587, 450, 272 and 152 bp in size. The strain of Brucella from sheep was characterized by absence of the 1,682-bp fragment, what distinguishes B. ovis from other species. MLVA analysis revealed the same VNTR profile of the isolates from cattle, wild boars and hares as reference strain B. suis by 2 Thomsen: VNTR [2-4-8-14-6-1-5-2]. The strain isolated from pigs had different characteristic: VNTR [2-5-8-9-5-1-5-5]. The B. ovis strain had the same profile as the reference strain B. ovis 63/290: VNTR [3-5-2-10-1-1-5-2].

It was shown previously that population of animals in Poland is free from infections caused by B. abortus and B. melitensis (Pilaszek et al. 2000, Szulowski et al. 2012). The only significant problem are infections caused by B. suis biovar 2. This biovar can affect both wild animals, which constitute a reservoir of this microorganism, and domestic pigs and cattle (EFSA 2009). The previous investigations revealed that the prevalence of anti-Brucella antibodies in wild boars in Poland was about 12%, exceeding even 20% in some regions, whereas in hares was approximately 1% (Pilaszek et al. 2000).

Fenotypic characteristics of the strains show that they are typical and identical to those presented by Alton et al. (1988). At the same time a full correlation between fenotypic and PCR profiles were observed leaving no doubt for identification. The only differences concerned the strain isolated from pigs (boars). While phenotypic characteristics of this strain were identical with those presented by strains isolated from wild boars and hares, the MLVA analysis revealed the VNTR profile characteristic for Iberian strains of B. suis biovar 2, isolated in Spain and Portugal from pigs and wild boars, what is shown in a database “MLVA-NET for Brucella” (http://mlva.upsud.fr/brucella). The result was fully justified as boars originated from Iberian Peninsula and infection was confirmed at quarantine station (Szulowski et al. 2011).

**References**


