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Short communication

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 routine test dilution – RTD and 10⁴ × RTD and R/C – RTD).

Molecular examination. Additionally, molecular methods – a multiplex PCR assay (Bruce – ladder) which enables to identify *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 phenotypic 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* bv 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).

Phenotypic 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 phenotypic 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

- Alton GG, Jones LM, Angus RD, Verger JM (1988) Techniques for the brucellosis laboratory. 1st ed., INRA, Paris.
- EFSA Scientific Opinion of the Panel on Animal Health and Welfare. Porcine brucellosis (*Brucella suis*) (2009) The EFSA Journal 1144: 1-112.
- Le Fleche P, Jacques I, Grayon M, Al Dahouk S, Bouchon P, Denoeud 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, Cloeckert 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* species including the vaccine strains. J Clin Microbiol 46: 3484-3487.
- Pilaszek J, Szulowski K, Iwaniak W (2000) Epidemiological condition of animal brucellosis in Poland. Med Weter 56: 363-366.
- Szulowski K, Iwaniak W, Weiner M, Złotnicka J, Szymajda M, Zaręba Z, Czępińska H (2012) Diagnosis and epidemiological condition of bovine brucellosis in Poland. Med Weter 68: 110-113.
- Szulowski K, Iwaniak W, Złotnicka J, Weiner M, Zaręba Z, Czępińska H (2011) International trade – a potential source of brucellosis in pigs. Med Weter 67: 64-66.