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Short communication

Determination of polymorphism of IS-1311 sequence in *Mycobacterium avium* subspecies *paratuberculosis* strains isolated from milk samples

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Abstract

The study was aimed at the genetic typing of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) with PCR-REA method based on the polymorphism of IS-1311 sequence. MAP strains were isolated from milk samples collected from cows free of disease symptoms and anti-MAP antibodies. Samples of udder milk were collected from 310 cows originating from the herd with a low seroprevalence of paratuberculosis; every 5 samples were pooled. The polymorphism typing of the IS-1311 sequence of the isolated strains demonstrated the presence of two types of the mycobacterium: sheep and cattle ones, of which the bovine type constituted 98.5%.

Key words: *Mycobacterium avium* subsp. *paratuberculosis*, milk, IS-1311 polymorphism

Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) induces chronic, hypertrophic jejunoileitis in ruminants, called paratuberculosis or Johne's disease (JD). The JD spreads in herds of dairy cattle across all continents (Nielsen and Toft 2008, Geraghty et al. 2014) and causes significant economic losses (Hasonova and Pavlik 2006). Today, in herds of dairy cattle, this disease occurs more frequently in the sub-clinical form (Brander et al. 2013). Investigations conducted so far have proved that one cow with clinical symptoms of JD corresponds to 9 cows with the

sub-clinical form and to 10-15 infected animals (Cocito et al. 1994). The presence of mycobacteria in milk increases the risk of consumer exposure to these pathogens, especially that they are not completely inactivated during industrial pasteurization (Grant 2003). The real spread ratio of paratuberculosis in herds of dairy cattle is unknown; likewise information is missing on the genetic diversity of MAP strains occurring in milk. This study was aimed at determining the genetic type of MAP mycobacterium, isolated from milk samples, based on the polymorphism of the IS-1311.

Materials and Methods

Milk samples (100 mL) were collected from 310 dairy cows over the age of 2 years, without disease symptoms nor positive serological results. Once transferred into the laboratory, every 5 samples of milk were pooled by collecting 10 mL of milk from individual samples. Thus obtained 62 laboratory samples, with the volume of 50 mL each, were subjected to the procedure described earlier by Szteyn (2006). The prepared suspension was seeded onto three slants of the HEYM medium (Herrold's egg yolk medium) with the addition of mycobactin J. The inoculated cultures were incubated for 16 weeks. The selected colonies, typical of the genus *Mycobacteria*, were subjected to the procedure of DNA isolation using a QIAamp DNA Mini Kit (Qiagen) according to the producer's procedure.

To confirm DNA/MAP presence, the IS-900 fragment was detected in the isolated genetic material with the PCR method. Reaction mixture (25 µL) was prepared using Hot-Start PCR StartWarm (A&A Biotechnology) and contained: 12.5 µL of PCR Master Mix, 2 µL of a primer mix P90+ and P91 (Giese and Ahrens 2000), 9.5 µL of DNA-free water, and 0.5 µL of isolated DNA. The reaction was conducted in a Mastercycler pro thermocycler (Eppendorf) under conditions described previously (Giese and Ahrens 2000). The results were read out and saved using a kit for gel documentation GelDoc – UVP (BioRad). A MAP strain ATCC-BAA-968 served as the positive control. The samples with the confirmed presence of the IS-900 sequence were subjected to further analysis in order to determine IS-1311 sequence polymorphism as described by Whittington (2004). Mycobacterium type was determined based on the number and size of the obtained bands.

Results and Discussion

The first stage of the study including the standard culture of mycobacteria on HEYM medium, which offers better conditions from MAP isolation of milk samples (Wiszniewska et al. 2004), resulted in the growth of colonies typical of *Mycobacterium sp.* In 35 out of the 62 cultures of pooled milk samples we observed the growth of colonies with characteristic phenotypic traits. After DNA isolation from the culture material, MAP prevalence was confirmed based on the presence of the IS-900 sequence in 24.1% of all milk samples. Polymorphism analysis of the insertion sequence IS-1311 demonstrated the presence of two types of mycobacterium. In 14 isolates, the genetic material was typical of the C type (cattle), and in one

case the arrangement of bands was different and typical of the S type (sheep). The molecular characteristics of strains is necessary for a better understanding of disease epidemiology and for developing effective methods of its prevention and eradication. The applied molecular techniques were based on the use of specific sequences present in MAP genome. In our study, we applied the PCR-REA technique for polymorphism typing of the IS-1311 sequence. Although this insertion sequence is not typical only for MAP because it shows 85% homology with IS-1245 (Collins et al. 1997, Whittington et al. 1998), it is subjected to enzymatic digestion together with IS-900 sequence. It is common knowledge that IS-1311 exhibits a single nucleotide polymorphism (SNP) at the position 223. Based on that, by using the PCR-REA method it is feasible to distinguish the C type of MAP from the S type and the intermediate B type referred to as Indian Bison (Sevilla et al. 2005). In our study, only one isolate exhibited MAP traits of the S type, while the remaining 14 isolates were MAPs of C type. Determinations conducted with the PCR-REA method on strains from various geographical regions (India, Canada, USA, Spain, and Portugal) demonstrated that the isolated MAPs were only of the C type (Sing et al. 2015). Australian studies (Whittington et al. 2000) carried out on a significantly larger material with two methods, RFLP and PCR-REA, also revealed preponderance of the C over the S type of MAP. However, the first one was isolated from samples collected not only from cattle but also from sheep and alpaca, and from two patients suffering from Leśniowski-Crohn disease. According to Stewenson (2015), determination of the genetic diversity of MAP is significant for understanding of disease transmission and development; such information is also indispensable for the development of more effective vaccines considering food as a MAP transmission vector.

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