



Evaluation of associations between genotypes of *Mycobacterium avium* subsp. *paratuberculosis* and presence of intestinal lesions characteristic of paratuberculosis



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ABSTRACT

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of paratuberculosis affecting ruminants worldwide. Depending on the MAP-Type (MAP-C or MAP-S, cattle or sheep type), strains differ in virulence and host preference. There is not yet any strong evidence indicating that individual field strains of the same MAP-subgroup exhibit differences in virulence.

The aim of this study was to evaluate a potential association between the genotype of individual field strains belonging to the MAP-C group and the presence of macroscopic intestinal lesions characteristic of paratuberculosis in the infected animals.

88 MAP-C isolates were sampled from clinically healthy cows at slaughter. Cows were grouped as A (n = 46) with, and B (n = 42) without macroscopic intestinal lesions. Sampled cows from both the A and B groups came from different farms and had a similar age distribution. MAP isolates were characterized by MIRU-VNTR and IS900-RFLP analysis. Resulting genotypes were examined for an association with the presence of macroscopic intestinal lesions characteristic of paratuberculosis.

MAP isolates from groups A and B exhibited similar strain diversity: 20 and 18 combined genotypes, altogether 32 genotypes. Six of these genotypes were detected in both groups. Although no association was found between individual combined genotypes and presence of macroscopic intestinal lesions, IS900-RFLP-(*BstEII*)-Type-C1 (the most common type worldwide) was found more often in group A ($p < 0.01$).

The data give only weak indication for the existence of differences in virulence among MAP-cattle type isolates. Differences in the development and severity of lesions may rather depend on unknown host factors or inoculation dose. Virulence properties of IS900-RFLP-(*BstEII*)-Type-C1 isolates should be examined in more detail.

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1. Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the etiologic agent of chronic granulomatous enteritis and lymphadenitis in ruminant species – known commonly as paratuberculosis or Johne's disease, exerts a tremendous economic burden especially on global dairy cattle health. After a long incubation period, this disease leads to emaciation, therapy resistant diarrhea

and eventually to death. Paratuberculosis also affects beef cattle, sheep, goats, camelids as well as farmed and wild living deer (Gosh et al., 2012; Sevilla et al., 2007; Verdugo et al., 2014; Whittington et al., 2000). MAP is divided into two major groups based on phenotypic and genotypic characteristics, designated as MAP-S (sheep)-type or Type I/III and as MAP-C (cattle)-type or Type II (reviewed by Biet et al., 2012; De Juan et al., 2006a, 2006b). These types exhibit different host distribution and culture phenotypes (Stevenson et al., 2002; Stevenson, 2015; Whittington et al., 2011). Although MAP-C strains were first isolated from cattle they also infect numerous other ruminants, and have been detected in non-ruminants and humans. MAP-S/Type I strains have been isolated almost exclusively from sheep. MAP-S/Type III strains (intermediate type) are closely related to Type-I strains (Gosh et al., 2012;

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Möbius et al., 2015), but show a broader host range, need a shorter time for cultivation than Type I, and exhibit unique IS900-RFLP and Pulsed-Field Gel Electrophoresis (PFGE) patterns (Biet et al., 2012; Cousins et al., 2000; De Juan et al., 2005; Möbius et al., 2009; Sevilla et al., 2007; Stevenson et al., 2002; Whittington et al., 2000).

Within the major MAP type groups, isolates can be discriminated by a combination of several genotyping methods (Fritsch et al., 2012; Möbius et al., 2008; Thibault et al., 2008). This is useful for transmission studies (Fritsch et al., 2012), for characterization of strains applied in pathogenesis studies and for identification of phenotypic associations (Borrmann et al., 2011).

MAP strains were regarded as a relatively homogeneous population; however, recent data suggest that the strains belonging to the two main MAP lineages are genetically and functionally more diverse than previously recognized (Castellanos et al., 2009; Möbius et al., 2015; Stevenson, 2015). Several phenotypic features that are likely to be associated with putative virulence properties are different between MAP-C and MAP-S: growth rate (Collins et al., 1990), cell invasion, cytokine induction and persistence in macrophages (Borrmann et al., 2011; Gollnick et al., 2007; Janagama et al., 2006), as well as induced pathological responses in lambs and red deer (Verna et al., 2007; Mackintosh et al., 2007). In contrast to these inter-subgroup-MAP-type differences, until now there is no clear evidence with regard to intra-subgroup-MAP-type differences of virulence properties. Only Verna et al. (2007) found differences in lesion intensity developed after experimental infection of lambs using heterogeneous bovine field isolates from Argentina and Spain, possibly caused by subtle combinations of specific mycobacterial gene expressions facilitating host/cell interplay. However, a relatively low degree of genetic diversity observed among isolates belonging to MAP-C was confirmed by whole genome sequencing and SNP analysis (Ahlstrom et al., 2015; Möbius et al., 2015; Stevenson, 2015).

The aim of this study was to find differences in virulence between individual MAP field strains belonging to the MAP-C (Type II) group. An indirect method was chosen: MAP isolates originating from slaughtered cattle with (group A) or without (group B) macroscopic intestinal lesions characteristic of paratuberculosis were genotyped by Mycobacterial Interspaced Repetitive Units – Variable-Number of Tandem Repeat analysis (MIRU-

VNTR) and also by Restriction Fragment Length Polymorphisms analysis coupled with hybridization to IS900 (IS900-RFLP). The distribution of detected genotypes in cattle with or without lesions was then evaluated statistically.

2. Material and methods

2.1. Origin of bacterial strains

Bacterial strains were sampled in 2008 in the context of a study that estimated the prevalence of MAP infection by sampling clinically healthy cattle at slaughter (Elze, 2009; Elze et al., 2013). Altogether 88 isolates cultured from jejunum, ileum, mesenteric or caecal lymph nodes of animals with macroscopic intestinal lesions that were characteristic of paratuberculosis (group A, n=46), and from animals without macroscopic intestinal lesions (group B, n=42) were included in this study. All cattle came from different farms of four Federal States in Germany and were female. The average age was 4.6 years (1690 days) in group A and 5.1 years (1855 days) in group B with a minimum age of 2.2 and 2.3 years, respectively. The sampled cattle originated from two regional slaughterhouses in Germany. The following gross changes of intestinal morphology were used as selection criteria for animals of group A: thickening of the intestinal wall, thick rugose mucosa, granular appearance of the intestinal mucosa and thickened mesenteric lymphatic vessels (see Fig. 1). To be included in group A, cattle had to fulfil at least one of these criteria. Cattle of group B were sampled systematically according to a predefined sampling scheme. In slaughterhouse 1: every tenth animal was sampled, altogether 100 cattle; in slaughterhouse 2: every fifth animal was sampled – altogether 50 cattle. Sampling was started at random.

2.2. Isolation of strains

Isolation of MAP strains and confirmation of species identity was done as described by Elze et al. (2013). Briefly, samples of 1 g were prepared, disrupted and decontaminated using 0.9% hexadecylpyridinium chloride (HPC, Merck, Germany) for 24 h. The material was inoculated on four slants of Herrold's egg yolk medium with mycobactin J (HEYM, Becton Dickinson, USA) and incubated at 37 °C for 16 weeks. Colony growth was examined

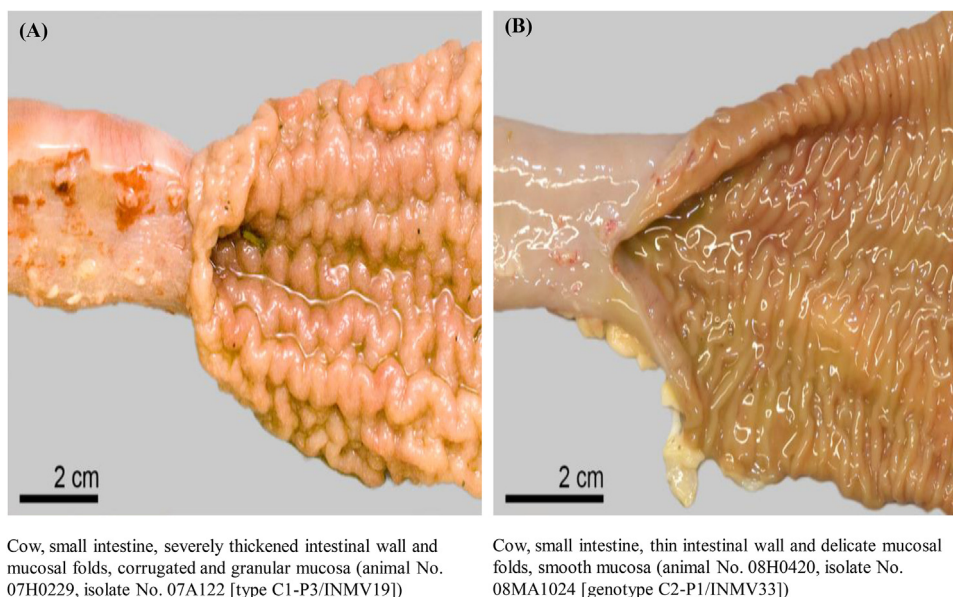


Fig. 1. Small intestine from cows with (A) and without (B) macroscopic intestinal lesions characteristic of paratuberculosis.

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