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Serological and molecular comparison of *Mannheimia haemolytica* and *Pasteurella trehalosi* strains isolated from wild and domestic ruminants in the French Alps

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Abstract

Over a period of 17 years, 84 bacterial isolates identified as *Mannheimia haemolytica* or *M. glucosida*, and 52 isolates identified as *Pasteurella trehalosi* were detected in the lungs of domestic and wild ruminants in the French Alps. The isolates were serotyped according to their surface capsular antigens, and those sharing common antigens were further characterized by pulsed field gel electrophoresis. The results showed that the bacterial isolates included in the study clustered according to the host species from which they were isolated. These findings indicate that the transmission of serotypes of *M. haemolytica*, *M. glucosida* or *P. trehalosi* from an animal host in which they are common to another species sharing the same geographical space may be a rare epidemiological event.
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1. Introduction

Mannheimia haemolytica and *Pasteurella trehalosi* are well-known pathogens of ruminants world-wide (Franck, 1989; Odendaal and Henton, 1995; Odugbo et al., 2004). Formerly, these two species belonged to a single species, *P. haemolytica*, in which two biochemical types were recognized and subsequently designated as biotype A and T, the letters standing for arabinose or trehalose fermentation, respectively (Adlam, 1989; Mutters et al., 1989). Recently, the strains belonging to the T biotype have been included in a new species, *P. trehalosi*, and the taxonomy of *M. haemolytica* has been further modified (Angen et al., 1997, 1999a,b; Sneath and Stevens, 1990). Both species can be serotyped by indirect haemagglutination according to their capsular antigens. Four and 12 (Younan and Fodor, 1995) serotypes have now been defined in *P. trehalosi* and *M. haemolytica*, respectively, and the ancient A11 serotype of *P. haemolytica* is now recognized as a separate species, *M. glucosida* (Angen et al., 1999a,b). Each isolate of *M. haemolytica* or *P. trehalosi* continues to be designated by a combination with a letter and number indicating biotype and serotype.

Bovine pasteurellosis mostly involves *M. haemolytica* type A1 (Franck, 1989; Odendaal and Henton, 1995), whereas *M. haemolytica* A2, and *P. trehalosi* T3, T4,

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and T10 mainly cause systemic pasteurellosis and/or septicaemia in lambs (Gilmour and Gilmour, 1989; Richard et al., 1986; Sanchis et al., 1991) or wild ruminants (Richard et al., 1992; Snipes et al., 1992). However, strains belonging to the same serotype have been isolated from different animal species. For example, *M. haemolytica* serotype A1 can be isolated from cattle, sheep and goats (Kodjo et al., 1999; Sanchis et al., 1988, 1991) and *P. trehalosi* type T3 has been isolated from sheep, goats and wild ruminants such as the chamois (*Rupicapra rupicapra*) (Fodor et al., 1996; Foreyt et al., 1996; Kodjo et al., 1999; Richard et al., 1992; Snipes et al., 1992).

Several serological studies have suggested that interspecies transmission of *M. haemolytica* may occur, with domestic animals transferring infection to free-living animals or vice versa. Callan et al. (1991) and Foreyt et al. (1982, 1996) demonstrated that under controlled experimental conditions, close contact between healthy domestic sheep and healthy rocky mountains bighorn sheep (*Ovis canadensis*) or Dall sheep (*Ovis dalli dalli*) may result in fatal pneumonia in the wild animals caused by *M. haemolytica* or *P. multocida* originating from the domestic ruminants.

In France, a long period of observation has shown that wild ungulates, mainly young chamois, are very sensitive to pasteurellosis. The presence of respiratory tract pathology from which *Pasteurella* species are recovered in those domestic and free-living ruminants which cohabit the high mountain pastures in the French Alps suggests a possible interspecies transmission. The aim of the present study was to characterise serologically and compare by pulsed field gel electrophoresis samples of *M. haemolytica* and *P. trehalosi* isolated from domestic ruminants with those isolated from free-living animals in the Alps over a period of more than 17 years.

2. Materials and methods

2.1. Bacterial strains

A total of 136 isolates were received in our laboratory for further analysis over a period over 17 years. Eighty-four were characterised as *Mannheimia* species (*M. haemolytica* and/or *M. glucosida*) and 52 as *P. trehalosi*. They were isolated either from domestic ruminants – mostly sheep and goats – (60 and 21 strains of *Mannheimia* species and *P. trehalose*, respectively), or wild ruminants – mostly chamois (*Rupicapra rupicapra*) – (24 and 31 isolates of *Mannheimia* species and *P. trehalosi*, respectively). The chamois came from the French Alps (Parc national de la Vannoise and Parc national des Ecrins, both in Savoie).

The field isolates were collected when domestic ruminants were on high mountain pasture (spring, summer and early autumn) and recovered from the lungs of dead free-living animals with post-mortem lesions of pneumonia suggesting pasteurellosis. After identification, all isolates were kept at −70 °C in brain heart infusion broth with 15% glycerol or were kept freeze-dried until required.

2.2. Biochemical identification of species

All isolates were identified using conventional bacteriological methods and were further characterised using API micro standardised strips (API Zym, API 20NE, API 20E and the API 50 CH, Biomérieux). Isolates were identified to species according to the methods of Cowan (1974); Mannheim (1984); Gilmour and Gilmour (1989) and Holt et al. (1994).

2.3. Serotyping

Isolates were serotyped using indirect haemagglutination with bovine red blood cells and specific *P. trehalosi* and *M. haemolytica* serotypes antisera.

2.4. Pulsed field gel electrophoresis

Total genomic DNA of each *M. haemolytica* and *P. trehalosi* isolate was prepared in 1% low-melting-temperature agarose (SeaPlaque GTG, FMC Bioproducts) according to a specific method previously described for pulsed field gel electrophoresis (PFGE) typing of *M. haemolytica* or *P. trehalosi* (Kodjo et al., 1999). Briefly, the DNA samples were digested overnight at 37 °C in 300 μL of the appropriate restriction buffer containing 10U of *Sal*I endonuclease (Roche Applied Science). The digested DNAs were separated by PFGE in a contour-clamped homogeneous electric field with the CHEF DRIII apparatus (Bio-Rad). Samples were loaded in 1% molecular biology-grade agarose gel (Fast Lane, Bioproducts) prepared by dissolution in 0.5× TBE buffer and were run in the same buffer at 6 V/cm at 12 °C with pulse times of 5–20 s for 20 h. The gels were stained in 1 μg/mL ethidium bromide aqueous solution for 30 min and photographed on a UV transilluminator. DNA fragment sizes were determined with the Taxotron software (Institut Pasteur) by interpolation from the sizes of Pulse Marker (Sigma–Aldrich) which was used as molecular size standard. For a simple computer database editing, each strain was given a species name, a capsular type and a PFGE pattern.

3. Results

3.1. Bacterial species identification or confirmation

After 48 h of aerobic incubation at 37 °C, colonies were approximately 2 mm diameter, smooth, shiny and

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