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Transmission dynamics of *Mannheimia haemolytica* in newly-received beef bulls at fattening operations

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ABSTRACT

The primary objective of this study was to determine, at the lung level, whether single or multiple clones of *Mannheimia haemolytica* are present within a pen during a bovine respiratory disease (BRD) episode. A secondary objective was to assess whether *M. haemolytica* isolates obtained from nasal swabs (NS) are identical to those isolated deeper within the respiratory tract. Sixteen BRD episodes that naturally occurred in 12 pens of eight to 12 bulls ($n = 112$) newly-received at three fattening operations were investigated. One hundred and seventy five *M. haemolytica* isolates were collected from 239 pairs of trans-tracheal aspirations (TTA) and NS performed during these 16 BRD episodes. *M. haemolytica* isolates were characterized by pulsed-field gel electrophoresis (PFGE). PFGE types obtained from NS and TTA were then compared. *M. haemolytica* was isolated during 14 BRD episodes. Two to three different clones of *M. haemolytica* were recovered during 10 episodes whereas only one clone was recovered in four episodes. A moderate agreement (kappa = 0.50) between NS and TTA for *M. haemolytica* isolation was observed. Identical PFGE types were only observed in 77% of matched NS-TTA pairs. The significant within-pen diversity of *M. haemolytica* during BRD episodes indicates that the disease is not primarily due to the spread of a single virulent clone among cattle and highlights the importance of predisposing factors that enable the resident flora to overcome the cattle's immune system. The results also demonstrate that isolates recovered from NS are not always representative of the isolates present deeper within the respiratory tract.

1. Introduction

Bovine respiratory disease (BRD) is the most prevalent disease in cattle entering fattening operations (Assie et al., 2009; Smith, 1998). *Mannheimia haemolytica* is the principal bacterium implicated in BRD and it is generally accepted that its control would markedly reduce the prevalence of BRD in fattening operations (Rice et al., 2007).

A good understanding of the transmission dynamics of *M. haemolytica* is needed to adapt control measures during BRD episodes (Miles, 2009). However, to date, little information on the transmission dynamics of this bacterium is available and it is not clear whether *M. haemolytica*-associated BRD episodes are due to predisposing factors that enable the resident flora to overcome the cattle's immune system or due to the contagious spread of a single virulent clone among penmates or due to both (Rice et al., 2007; Taylor et al., 2010b).

Molecular typing methods provide tools to investigate the transmission dynamics of *M. haemolytica*. Among these methods, pulsed-field gel electrophoresis (PFGE) appears to be well adapted to monitor the transmission dynamics of *M. haemolytica* at the BRD episode level due to its high discriminatory power and repeatability (Klima et al., 2010; Kodjo et al., 1999). It can be applied on isolates recovered from the upper and/or lower respiratory tracts. Because

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the sampling of the nasal flora is easier to perform than that of the lung, it is generally preferred (Klima et al., 2011; Purdy et al., 1993). However, further investigation is needed to determine whether *M. haemolytica* isolates obtained from the nasal cavities are representative of the isolates present deeper within the respiratory tract (Taylor et al., 2010b).

The objectives of this study were therefore, firstly, to characterize by PFGE the *M. haemolytica* isolates collected from the lower respiratory tracts of bulls during BRD episodes to determine whether single or multiple clones of *M. haemolytica* are present within a pen and, secondly, to assess whether *M. haemolytica* isolates recovered from nasal swabs (NS) are identical to those isolated deeper within the respiratory tract.

2. Materials and methods

All procedures in the present experiment were performed in accordance with the European directive and the French regulation and conform to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

2.1. Animals

One hundred and twelve bulls (initial body weight ± SD = 346 ± 36 kg) were observed for 40 days following their arrival at three French fattening operations between November 2007 and March 2008 (Table 1). Bulls came from multiple sources (n = 43 farms of origin) and were purchased at auction markets. After purchase, bulls were transported by truck over a mean distance of 329 ± 104 km (range = 70–515 km) to a central facility where they were sorted into 12 groups of eight to 12 bulls based on body weight (Table 1). The size of the group (eight to 12 bulls) was determined by the size of the bulls' pen in the fattening operations, which ranged from 30 to 48 m^2. Bulls were allowed free access to hay and water at the central facility for 36 to 48 h. During this period, they were in contact with others bulls present at the central facility. Then, bulls were transported by truck for travel distances less than 50 km to the fattening operations. None of the bulls received any vaccine or antibiotics at entry. During the study period, each group of bulls was housed in a pen separated from the other bulls fed in the fattening operation by fences that allowed nose-to-nose contact. Bulls were fed with a total mixed ration formulated to meet the French National Institute for Agriculture Research recommendations (Garcia et al., 2007). Feed was mixed and delivered once daily at 9 a.m. Throughout the study period, animals had unlimited access to water.

2.2. Study design

During the 40-day-study period, owners observed twice daily all bulls for the detection of the following signs: depression, decreased rumen fill compared with penmates, nasal or ocular discharge, cough and increased respiratory rate. As soon as the owner detected a bull displaying at least one of the above signs in a pen under study, a veterinarian with experience in cattle disease diagnostics restrained, one by one, each bull housed in the pen including the in-contact apparently healthy bulls in a conventional cattle handling chute, to perform a close physical examination, a NS sample and a transtracheal aspiration (TTA).

Bulls with a rectal temperature ≥ 39.7 °C and, at least, one other sign of respiratory tract disease were diagnosed as clinically BRD affected and received a single subcutaneous injection (2 mL per 15 kg of body weight) of a product containing 16.5 mg/mL of flunixin meglumin and 300 mg/mL of florfenicol (Resflor, MSD, Angers, France).

Physical examinations and clinical samples (NS and TTA) were then repeated every three days on non-previously treated bulls until the end of the BRD episode *i.e.* until no new BRD affected animal was detected.

2.3. Sampling procedures and bacteriology

Prior to NS sampling, the nostril was disinfected using 90% alcohol. A guarded swab (Dryswab Veterinary Laryngeal, Medical Wire and Equipment, Corsham, England) was used to sample the nasal cavities. This swab was enclosed in a sterile plastic sleeve in order to reduce contamination while it was introduced through the nostril to a depth of approximately 20 cm (dorsal conchae). At this point, the swab was exposed by withdrawing the sleeve and it was moved back and forth several times against the nasal mucosae as previously described (Allen et al., 1991).

Table 1
Characteristics of 12 pens of newly-received bulls at three fattening operations.

Fattening operation no.	Date of arrival	Pen no.	No. of bulls per pen	No. of different origins per pen	Mean body weight at arrival ± SD, kg
1	16/11/2007	1	9	1	314 ± 28
		2	9	2	326 ± 26
	17/01/2008	3	9	7	378 ± 14
		4	9	6	355 ± 43
2	21/11/2007	5	8	6	330 ± 19
		6	8	5	334 ± 20
		7	8	5	350 ± 25
	06/12/2007	8	8	5	359 ± 22
		9	8	2	364 ± 27
3	27/11/2007	10	12	3	379 ± 34
		11	12	4	350 ± 31
	11/01/2008	12	12	9	303 ± 19