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Short communication

Mannheimia haemolytica serotype A1 exhibits differential pathogenicity in two related species, *Ovis canadensis* and *Ovis aries*

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ABSTRACT

Mannheimia haemolytica causes pneumonia in both bighorn sheep (BHS, *Ovis canadensis*) and domestic sheep (DS, *Ovis aries*). Under experimental conditions, co-pasturing of BHS and DS results in fatal pneumonia in BHS. It is conceivable that certain serotypes of *M. haemolytica* carried by DS are non-pathogenic to them, but lethal for BHS. *M. haemolytica* serotypes A1 and A2 are carried by DS in the nasopharynx. However, it is the serotype A2 that predominantly causes pneumonia in DS. The objectives of this study were to determine whether serotype A1 exhibits differential pathogenicity to BHS and DS, and to determine whether leukotoxin (Lkt) secreted by this organism is its primary virulence factor. Three groups each of BHS and DS were intra-tracheally administered either 1×10^9 cfu of serotype A1 wild-type (*lktA*-Wt group), Lkt-deletion mutant of serotype A1-(*lktA*-Mt group), or saline (control group), respectively. In the *lktA*-Wt groups, all four BHS died within 48 h while none of the DS died during the 2-week study period. In the *lktA*-Mt groups, none of the BHS or DS died. In the control groups, one DS died due to an unrelated cause. Necropsy and histopathological findings revealed that death of BHS in the *lktA*-Wt group was due to bilateral, fibrinohemorrhagic pneumonia. Although the A1-Mt-inoculated BHS were clinically normal, on necropsy, lungs of two BHS showed varying degrees of mild chronic pneumonia. These results indicate that *M. haemolytica* serotype A1 is non-pathogenic to DS, but highly lethal to BHS, and that Lkt is the primary virulence factor of *M. haemolytica*.

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1. Introduction

Mannheimia (*Pasteurella*) *haemolytica* is the primary bacterial pathogen causing severe fibrinonecrotic pneumonia in cattle, goats, bighorn sheep (BHS, *Ovis canadensis*) and domestic sheep (DS, *Ovis aries*; Frank, 1989; Mosier, 1997; Brogden et al., 1998; Miller, 2001). However, BHS are much more susceptible to pneumonic pasteurellosis than DS (Foreyt, 1994). Although multiple *M. haemolytica* serotypes including A1 and A2 are present in the upper respiratory tract of healthy ruminants, serotype A1 is the primary cause of bovine pneumonic pasteurellosis, while serotype A2 is the primary cause of pneumonia in DS (Higlander, 2001; Odugbo et al., 2004). BHS have been shown to be susceptible to serotype A2 (Foreyt et al., 1994).

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In five independent studies employing a total of 43 BHS, 41 BHS died of pneumonia following co-pasturing with DS (Foreyt and Jessup, 1982; Onderka and Wishart, 1988; Foreyt, 1989, 1994; Callan et al., 1991). It is conceivable that certain strains/serotypes of *M. haemolytica* carried by DS are non-pathogenic to them, but lethal for BHS. We hypothesized that at least some strains of serotype A1 are non-pathogenic to DS but highly fatal for BHS. Therefore, the first objective of this study was to determine the effects of intra-tracheal administration of a strain of *M. haemolytica* serotype A1 in BHS and DS.

M. haemolytica produces several virulence determinants, of which leukotoxin (Lkt) and lipopolysaccharide (LPS) are considered to be the most important ones in cattle (Frank, 1989; Higlander, 2001; Jeyaseelan et al., 2002). We desired to confirm that Lkt is the most important virulence determinant in the pathogenesis of *M. haemolytica*-caused pneumonia in BHS and DS as well. Therefore the second objective of this study was to compare the effects of intra-tracheal administration of an Lkt-deletion mutant (Mt) with that of the wild-type (Wt) *M. haemolytica* serotype A1 in BHS and DS.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Development and characterization of the leukotoxin A gene (*lktA*) deletion mutant (Mt) from *M. haemolytica* serotype A1 strain 89010807N (*lktA*-Wt) has been described by us (KDC) previously (Murphy et al., 1995). The *lktA*-Wt and *lktA*-Mt were used in the animal challenge study. Bacteria were grown overnight at 37 °C in brain-heart infusion agar (BHI) supplemented with 5% sheep blood (Remel, Lenexa, KS). *M. haemolytica lktA*-Mt strain was grown in culture plates containing BHI supplemented with 10 μg/ml ampicillin (Bioline, Randolph, MA). Both the *lktA*-Wt and *lktA*-Mt strains cultured overnight in BHI-agar were washed twice with colorless RPMI 1640 (Invitrogen, Carlsbad, CA), resuspended in RPMI and the optical density (OD_{600}) of the suspension was adjusted to 0.4. The cultures were grown at 37 °C for additional 1–2 h to obtain exponential growth phase cultures (OD_{600} ~0.6, 1.2×10^{10} cfu).

2.2. Sheep challenge studies

All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC), at Washington State University before the onset of the study. Ten 2–4 years old mixed gender BHS were randomly divided into two groups of four each, and a third group of two animals which were kept as phosphate buffered saline (PBS)-inoculated controls. Twelve 2–6 years old female DS were divided into 3 groups of 4 each and housed separately. Pharyngeal swabs were taken from all the animals before bacterial inoculation to assess whether these animals harbor any *M. haemolytica* or *Pasteurella* species. The three groups of BHS and DS were intratracheally administered either 1×10^9 cfu (in 5 ml of sterile PBS, pH 7.2) of *lktA*-Wt (*lktA*-Wt group), *lktA*-Mt (*lktA*-Mt group), or saline (control group), respectively. Five milliliters of sterile PBS was given to each animal in each group to flush the trachea.

2.3. Clinical assessment and necropsy

The animals in each group were observed twice a day for up to 2 weeks for clinical signs of pneumonia such as anorexia, fever, lethargy, cough, dyspnoea and nasal discharge. The animals that died before the end of observation period were necropsied within 6 h, and lungs and other tissue samples were collected for both bacteriological and histopathological examinations. The animals that showed severe signs of pneumonia, and those that survived until the end of the study period were euthanized, necropsied and tissue samples collected. The lungs were removed from each animal and carefully examined for the signs of pneumonia. The degree of involvement of right and left lungs were noted as percent pneumonic scores. Pleuritis was noted as present or absent. Representative samples of pneumonic lesions and normal lung were prepared for histopathological examination (Odugbo et al., 2004).

2.4. Re-isolation of *M. haemolytica* from pneumonic lungs

Using aseptic techniques, fresh lung samples and swabs were obtained for isolation and characterization of bacteria including *M. haemolytica* and *P. trehalosi*. Serotyping of *M. haemolytica* isolates were performed using anti-serotype A1 and A2 specific sera kindly provided by Dr. Robert Briggs, National Animal Disease Center, Ames, IA.

3. Results

3.1. Evaluation of animals before the infection

Pharyngeal swabs were obtained from all animals before the administration of *lktA*-Wt or *lktA*-Mt strains to determine whether they carry *M. haemolytica* in their upper respiratory tract. The results of bacterial isolation are summarized in Table 1. Only three BHS, one from the *lktA*-Wt group, and two from the *lktA*-Mt group, were positive for *M. haemolytica* belonging to serotypes other than A1 or A2 (negative for reactivity with anti-A1 and anti-A2 sera). In contrast, all the BHS except two from the *lktA*-Wt group harbored *P. trehalosi* and/or untypable *Pasteurella* sp. in their pharyngeal region. Unlike BHS, seven DS, three from the *lktA*-Wt group, three from the *lktA*-Mt group, and one from the control group, were positive for *M. haemolytica* which were neither A1 nor A2. All DS except one from the control group were positive for *P. trehalosi* and/or *Pasteurella* sp. (Table 1). All the animals used in this study had very low Lkt-neutralizing antibody titers in their plasma as assessed by the MTT dye reduction cytotoxicity assay (Dassanayake et al., 2007). Culture supernatant of *M. haemolytica lktA*-Wt but not *lktA*-Mt showed typical cytotoxicity on BL-3 cells confirming that only *lktA*-Wt but not *lktA*-Mt strain used in this study produced Lkt (Fig. 1).

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