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Short communication

The upper respiratory tract is a natural reservoir of haemolytic *Mannheimia* species associated with ovine mastitis

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ABSTRACT

Lamb suckling has been suggested to be an important way of infecting a ewe's udder with different bacteria, including *Mannheimia haemolytica*. To test the potential role of lambs in transferring *Mannheimia* species to the ewe's udder, the restriction endonuclease cleavage patterns of isolates obtained from nasopharyngeal swabs were compared with those obtained from cases of mastitis. Sterile cotton swabs were used to collect nasopharyngeal samples from 50 ewes and 36 lambs from three flocks. *M. haemolytica* and *Mannheimia glucosida* as well as haemolytic *Mannheimia ruminalis*-like organisms were detected in the upper respiratory tract of lambs and ewes. Comparison of the restriction endonuclease cleavage patterns of the isolates suggested that the *M. haemolytica* isolates obtained from different milk samples from ewes with mastitis were more clonal than those obtained from the nasal swabs. However, some nasal isolates within both *Mannheimia* species had restriction endonuclease cleavage patterns identical to those obtained from milk samples from ewes with mastitis, indicating that lambs may have a role in transferring these organisms to the udder. More clonality was observed between the *M. glucosida* isolates than between *M. haemolytica* isolates.

1. Introduction

Mannheimia haemolytica is an opportunistically pathogenic organism and an obligate commensal of the upper respiratory tract of ruminants. It is the major organism involved in bovine respiratory disease and can cause ovine pneumonia and septicaemia (Boyce et al., 2004; Kirk and Glenn, 1996). Together with *Mannheimia glucosida*, they are known to be important causes of intramammary infection in sheep (Omaleki et al., 2010, 2011). Lamb suckling has been suggested as an important route for transferring *M. haemolytica* from the nasopharynx of lambs to the ewe's udder, as isolates obtained from the ovine respiratory tract had similar pathogenicity for ovine mammary tissue to those obtained from mammary secretions or mastitic milk (Fragkou et al., 2011). Studies have demonstrated that *M. haemolytica* cannot be isolated from the teat skin before lambing or after weaning, also indicating the likely role of lambs in transferring this organism from their oral cavity to the ewe's udder (Jones and Watkins, 1998). Colonisation of papilloma-like scabby lesions of the teat with different serotypes of *M. haemolytica*, including serotype A11 of the former [*P.*] *haemolytica*, latterly reclassified as *M. glucosida*, has also been reported, suggesting that these lesions can act as a source for contamination of the teat canal after lamb suckling (Burriel, 1997).

There are no published data investigating the genetic associations between isolates of *Mannheimia* species from the nasopharynx of sheep and those causing mastitis. Therefore, this study aimed to identify isolates of *M. haemolytica* and *M. glucosida* from the nasopharynx of sheep and compare the isolates previously found to be associated with ovine mastitis with those obtained from nasal swabs.

2. Materials and methods

Three Poll Dorset flocks were included in this study. Flock A had a history of clinical mastitis caused by *Mannheimia* species at a prevalence of up to 5% over the previous three years, flock J had had a mastitis outbreak in 2009 with 10% of lactating ewes affected (Omaleki et al., under revision), and flock K had had a prevalence of

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mastitis of less than 2% over the past three years. The three flocks were located in different regions of Victoria, Australia: flock A in the north-east, flock J in the north-west and flock K in central Victoria. Each of these flocks had 200–350 registered Poll Dorset ewes, with two of the three flocks having additional commercial ewes of different breeds on the property that were not mixed with the stud ewes. A limited number of rams and ewes had been transferred from flock K to flock A over the previous six years and a ram had been transferred from flock K to flock J.

Twenty isolates of *Mannheimia* species, including seven *M. haemolytica* and thirteen *M. glucosida*, obtained between 2006 and 2011 from cases of ovine mastitis in flock A, were included in this study. The isolates consisted of ten that have been described previously (Omaleki et al., 2010, 2012), as well as five new *M. haemolytica* and five new *M. glucosida* isolates (Table 1). Milk samples were collected from any ewe with clinical mastitis during lactation or at weaning in this flock during these years.

The mastitis samples from flock J included the isolates obtained from an outbreak of clinical mastitis caused by *M. haemolytica* in 2009 (Omaleki et al., under revision) and six more samples from sporadic cases of clinical mastitis during 2010 and 2011. Four of the more recent samples yielded cultivable bacteria, all identified as *M. haemolytica* (Table 1).

No milk samples from ewes with mastitis were available from flock K.

Sterile 20 cm long cotton swabs were used to obtain bilateral nasal samples from 50 ewes and 36 lambs in the three flocks (The University of Melbourne Animal Ethics Approval number 1011685.1). Ten ewes and 16 lambs in flock A were swabbed in December 2010, and 20 ewes and 10 lambs in each of flocks J and K were swabbed in July 2011. The swabs were used to make the primary inoculum on sheep blood agar plates (SBA) whilst on the farms. The plates were transferred to the laboratory at 4 °C, where streak dilution plates were completed and then incubated aerobically at 37 °C, overnight. Plates were visually assessed to identify *Pasteurellaceae*-like organisms. A single haemolytic, mucoid grey colony from each positive plate was subcultured and subjected to phenotypic testing and *rpo*B gene sequencing as described previously (Korczak et al., 2004; Omaleki et al., 2010). A further two colonies were picked and tested from two of the original plates in flock J from which *M. haemolytica* and *M. glucosida* were isolated.

Genomic DNA of the *M. haemolytica* and *M. glucosida* isolates was prepared in 1% low melting agarose and digested using the restriction endonuclease *Sal*I and the DNA fragments were separated in a 1% molecular biology grade agarose gel by PFGE using a procedure described previously (Gunawardana et al., 2000; Kodjo et al., 1999; Omaleki et al., 2012).

The similarities between restriction endonuclease cleavage patterns of each strain were calculated using the Dice similarity index (Dice, 1945). Dendrograms were constructed using the unweighted-pair group method using Bio-1D (Viber-Lourmat, France), allowing a 5% confidence interval for band matching.

Table 1
M. haemolytica and *M. glucosida* isolates from cases of ovine mastitis in flocks A and J included in this study.

Isolates	Year	Lactation status	Clinical status
M. haemolytica			
A8	2008	Lactating	Clinical
A9	2008	Lactating	Clinical
A14	2009	Weaning[b]	Clinical
A16	2009	Lactating	Clinical
A17	2009	Weaning[c]	4.5×10^{6}[a]
A20	2010	Weaning[b]	Clinical
A23	2011	Weaning[b]	Clinical
J1	2009	Lactating	Clinical
J2	2009	Lactating	Clinical
J3	2009	Lactating	Clinical
J4	2009	Lactating	Clinical
J5	2009	Lactating	Clinical
J6	2009	Lactating	Clinical
J8	2009	Lactating	Clinical
J9	2009	Dry	Clinical
J10	2009	Lactating	Clinical
J11	2009	Lactating	Clinical
J12	2009	Lactating	Clinical
J13	2009	Lactating	Clinical
J14	2009	Lactating	Clinical
J15	2009	Lactating	Clinical
J16	2009	Lactating	Clinical
J17	2009	Lactating	Clinical
J18	2010	Lactating	Clinical
J19	2010	Lactating	Clinical
J20	2011	Lactating	Clinical
J21	2011	Lactating	Clinical
M. glucosida			
A2	2006	Weaning[b]	Clinical
A3	2006	Lactating	Clinical
A4	2006	Lactating	Clinical
A5	2008	Weaning[c]	13.4×10^{6}[a]
A10	2009	Weaning[b]	Clinical
A11	2009	Weaning[b]	6.7×106[a]
A12	2009	Weaning[b]	Clinical
A13	2009	Weaning[b]	Clinical
A18	2010	Weaning[b]	Clinical
A19	2010	Weaning[b]	Clinical
A21	2010	Weaning[b]	Clinical
A22	2011	Weaning[b]	6.3×10^{6}[a]
A24	2011	Weaning[b]	4.5×10^{6}[a]

[a] These isolates were obtained from ewes with no clinical signs of mastitis at weaning. The numbers indicate the somatic cell counts per millilitre of milk.
[b] Samples collected at weaning in August of each year.
[c] Samples collected at weaning in December of each year.

3. Results

Haemolytic *Mannheimia* sp. isolates were obtained from 13 of the 26 nasal swabs collected in flock A (50%), with 4 of them identified as *M. haemolytica* and 9 as *M. glucosida* (31% and 69% of the haemolytic *Mannheimia* spp. isolates respectively) (Supplementary Table 1). The nasal swab isolates from this flock were designated with a lower case letter "a". In this flock, the *M. haemolytica* isolate a11, obtained from a nasal swab of a lamb in December 2010, was closely related to mastitis isolates A17 and A23, obtained in 2009 and 2011, respectively (one and two band differences, respectively) (Fig. 1), and were clustered together in the dendrogram (Supplementary Fig. 1). Two *M. glucosida* isolates obtained from nasal swabs of lambs in flock A shared an identical restriction endonuclease cleavage pattern with 6 isolates obtained from cases of mastitis over a 6 year investigation between 2006 and 2011 (nasal swab isolates a17 and a23 and mastitis isolates A2, A3, A4, A10, A21 and A24) (Fig. 2). Similarly, the nasal swab isolates a2 and a9, obtained from samples collected from ewes, shared an identical restriction endonuclease cleavage pattern with mastitis isolates A5 and A18, obtained in 2008 and 2010, respectively. Moreover, mastitis isolates A19 and A22, obtained in 2010 and 2011, respectively, had identical restriction endonuclease cleavage patterns to each other and to that of isolates isolates a1, a7, a8 and a10 from nasal swabs,and also to one of the two *M. glucosida* isolates obtained from nasal swabs in flock K, isolate k1.

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In flock J, 11 of the 30 nasal swab samples yielded isolates of haemolytic *Mannheimia* species (36.6%), with 7 identified as