Staphylococcus aureus mastitis in Texel sheep associated with suckling twins

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Short Communications

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THE incidence of clinical mastitis in sheep is usually below 5 per cent per year (Bergonier and others 2003, Contreras and others 2007). Clinical cases are most often associated with *Mannheimia haemolytica*, *Staphylococcus aureus* and coagulase-negative staphylococci (CNS), but *Streptococcus* species and enterobacteria have also been cultured from the udder of sheep (Lafi and others 1998, Bergonier and others 2003, Mørk and others 2007, Arsenault and others 2008). Factors that have been associated with the occurrence of clinical mastitis in sheep are parity, dystocia, breed, region and the number of lambs born (Larsgard and Vaabenoe 1993, Arsenault and others 2008, Waage and Vatn 2008). Suckling two or three lambs is associated with a greater mastitis risk than suckling only one lamb per ewe (Larsgard and Vaabenoe 1993, Waage and Vatn 2008). However, Arsenault and others (2008) reported an increased risk of mastitis in ewes suckling triplets, but found no difference in the risk of clinical mastitis between ewes with one or two lambs.

This short communication describes a flock of sheep with a relatively high incidence of clinical mastitis, associated with suckling more than one lamb per ewe. Bacteriological culture results showed that most cases were caused by *S aureus* or *M haemolytica*. The proportion of mastitis cases caused by *S aureus* was higher in sheep with twins than in sheep with a single lamb. Hypotheses about the biological mechanisms behind the observations are discussed.

A flock of 350 Texel sheep in the Netherlands had a farmer-reported mastitis incidence of approximately 8 per cent per year, which has been the case for at least the past 15 years. In order to improve udder health on the farm, intramammary treatment had been used at drying-off since 1999, and ewes with mastitis were separated from the flock and not bred again. However, these management measures did not decrease the incidence of mastitis. Lambs were born in March and the beginning of April. Approximately one month after lambing, the sheep with their lambs were pastured. The lambs were weaned at the age of three months. During the lactation period, the udder and milk of the sheep were examined by the farmer when any indication of possible udder problems, such as ewes showing general signs of illness, bleating lambs (a sign of hunger, as they were not getting enough milk) or obvious abnormalities of the udder, were observed. Clinical mastitis was defined as a warm, swollen udder, possibly with a change in colour to red or blue, and/or milk with an abnormal appearance. In 2007 and 2008, the date of each lambing and the number of lambs born, together with the age and parity of the ewe, were recorded. In these years, milk samples were collected aseptically from almost 50 per cent of the affected udder halves and frozen at approximately −20°C. Within two months, the milk samples were cultured for mastitis pathogens according to National Mastitis Council guidelines. The presence of one or more colony-forming units of a mastitis pathogen was considered a positive culture. If *S aureus* or *M haemolytica* was detected in a mixed culture with minor pathogens, the sample was considered positive for *S aureus* or *M haemolytica*. If both *S aureus* and *M haemolytica* were present in the same sample, it was considered a mixed infection.

TABLE 1: Incidence of mastitis and parity distribution of ewes in a Dutch sheep flock with mastitis problems

	Parity			
	1	2	≥3	Total
2007				
Number of ewes	152	71	125	348
Cases of mastitis	12	7	12	31
Incidence of mastitis (%)	7.9	9.9	9.6	8.9
2008				
Number of ewes	126	104	130	360
Cases of mastitis	8	7	11	26
Incidence of mastitis (%)	6.3	6.7	8.5	7.2

Logistic regression was used to test the hypothesis that the odds of clinical mastitis were equal for the ewes that suckled one lamb versus ewes suckling twins or triplets. This was tested in separate models for the years 2007 and 2008. Parity was included in the models to correct for possible confounding on this variable. Another logistic regression model was built on a subset of the data, including only the cases of mastitis caused by *S aureus* or *M haemolytica*, to test the hypothesis that the odds of infection with *S aureus* versus *M haemolytica* in ewes that suckled twins or triplets was equal to the odds in ewes suckling one lamb, corrected for parity as a possible confounder. In this model, the cases from both years were analysed together, because all the ewes that had mastitis in 2007 were culled and were therefore not included in the data for 2008.

The basic herd characteristics are shown in Table 1. Culture of 31 mastitis milk samples yielded *M haemolytica* (15 cases, 48 per cent) and *S aureus* (12 cases, 39 per cent). In one case (3 per cent), *M haemolytica* and *S aureus* were cultured from the same sample, and in another case (3 per cent) *Streptococcus uberis* and CNS were cultured together from one sample. In two cases (7 per cent), no pathogen was cultured. Table 2 shows the distribution of the different pathogens in samples from ewes with different number of lambs born. Mastitis occurred significantly more often in ewes with twins or triplets than in ewes with singletons in 2007 (odds ratio [OR] 4.1, 95 per cent confidence interval [CI] 1.5 to 11.2; P=0.005), but this difference was not significant in 2008 (OR 2.0, 95 per cent CI 0.8 to 4.7; P=0.12). Cases of mastitis caused by *M haemolytica* were evenly distributed between ewes with twins and single lambs, but mastitis caused by *S aureus* was seen almost exclusively in ewes suckling twins or triplets. The odds of infection with *S aureus* were greater in ewes suckling more than one lamb compared with ewes suckling one lamb (OR 9.5, 95 per cent CI 0.9 to 97.5; P=0.059).

In this study, the milk samples were frozen before culture. Villanueva and others (1991) observed an increase in the frequency of isolation of *S aureus* from cows' milk after freezing, but others did not find this (Schukken and others 1989, Murdough and others 1996, Artursson and others 2010). Freezing has been shown to decrease the amount of Gram-negative bacteria that could be cultured from cows' milk (Schukken and others 1989) and goats' milk (Sánchez and others 2003). Therefore, the number of *M haemolytica* isolates may have been underreported in the present study. However, because all the milk samples were frozen in this study, it is not expected that this possible

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TABLE 2: Results of culture of milk samples from ewes with clinical mastitis in a Dutch sheep flock, for ewes that had one, two or three lambs

	Number of lambs born			
	1	2	3	All
No clinical mastitis	303	340	9	652
Clinical mastitis	13	43	1	57
Staphylococcus aureus	1	10	1	12
Mannheimia haemolytica	7	8	0	15
S aureus + M haemolytica	0	1	0	1
Streptococcus uberis + CNS*	1	0	0	1
Culture-negative	0	2	0	2
Not cultured	4	22	0	26
Total	316	383	10	709

* CNS Coagulase-negative staphylococci

lower recovery of Gram-negative bacteria caused any bias on the effect of twins or triplets on the probability of infection with *M haemolytica* or *S aureus*.

Several hypotheses have been proposed to explain why ewes suckling twins are at greater risk of developing mastitis than ewes suckling one lamb. Damage to the teats and udder by vigorous and more frequent sucking have been suggested as possible causes of the higher incidence of clinical or subclinical mastitis (Larsgard and Vaabenoe 1993, Lafi and others 1998, Waage and Vatn 2008). This is supported by the fact that experimental infection with *M haemolytica* was facilitated by damage to the teats (Mavrogianni and others 2006, Fragkou and others 2007). A second possible explanation is an increased risk of teat contamination (Arsenault and others 2008). The presence of *M haemolytica* in the mouth of lambs, and transfer of these pathogens on to the teat skin, has been reported (Scott and Jones 1998, Gougoulis and others 2008).

The findings of this study show that in ewes with clinical mastitis, suckling twins was associated with culture of *S aureus* but not *M haemolytica*. No milk samples from clinically healthy animals were cultured, so it is not known whether twins increase the risk of infection with *S aureus* or the risk of developing clinical signs. Nevertheless, this study shows that *S aureus* somehow benefits from the suckling twins in the process of infecting the mammary gland or in causing clinical signs, or both, whereas *M haemolytica* is equally able to cause clinical mastitis in ewes with or without twin lambs. Differences in the pathogenesis of *S aureus* mastitis and *M haemolytica* mastitis are, however, small. Both pathogens are able to adhere to and invade mammary epithelial cells (Iturralde and others 1993, Hensen and others 2000, Aguilar and Iturralde 2001, Vilela and others 2004). The fact that *S aureus* seems to be more able to cause clinical mastitis in ewes suckling twins is therefore surprising. Both pathogens can be cultured from healthy teat skin (Bergonier and others 2003). *S aureus* is a normal udder skin commensal, whereas *M haemolytica* can often be found on the udder skin during the suckling period as a result from contamination from the mouths of the suckling lambs (Scott and Jones 1998). It is possible that *S aureus* infections need favourable conditions to result in mastitis. Damage to the teat skin and udder tissue by the frequent and rough sucking of twins may present such circumstances. Despite the fact that *M haemolytica* also benefits from damage to the teats (Fragkou and others 2007), this study seems to indicate that *M haemolytica* is less dependent upon these circumstances than *S aureus*. However, larger studies are needed to confirm the present findings, and to clarify the causal mechanisms behind these observations.

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