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Dynamics of intramammary infections in suckler ewes during early lactation

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ABSTRACT

Mastitis is an important problem in meat-producing sheep, but few studies have investigated the transmission dynamics of mastitis pathogens in these animals. The objective of this study was to describe the pathogens causing intramammary infections (IMI) in suckler ewes, their effect on somatic cell count, and the dynamics of these IMI in early lactation. We enrolled 15 sheep flocks early after lambing and selected ewes in each flock that were sampled twice with a 3-wk interval. Milk samples from both glands of each ewe were bacteriologically cultured, and somatic cell count was measured. Non-*aureus Staphylococcus* spp. were the most prevalent culture results. Somatic cell counts were most strongly increased in ewes infected with *Mannheimia haemolytica*, whereas staphylococci, including *Staphylococcus aureus*, were associated with a moderate increase in somatic cell count. The proportion of udder halves that remained culture-positive with *Staphylococcus* spp. during the 3-wk sampling interval was moderate, but *M. haemolytica* infections were stable during this time period. A substantial number of new infections were seen in the early lactation study period for non-*aureus Staphylococcus* spp., *Staph. aureus*, and *Corynebacterium* spp., but not for *M. haemolytica* or *Streptococcus* spp. The number of new IMI of *Staph. aureus* was associated with the number of *Staph. aureus*-infected udder halves in the flock at the first sampling moment, indicative of contagious transmission. Altogether, we show that substantial transmission happens in early lactation in suckler ewes, but that the dynamics differ between pathogen species. More research is needed to further describe transmission in different stages of lactation and to identify transmission routes, to develop effective interventions to control mastitis.

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INTRODUCTION

Mastitis in sheep causes substantial economic damage (Conington et al., 2008) and is a cause of impaired welfare (Munoz et al., 2018), mortality or culling (McLaren et al., 2020), and reduced growth in lambs (Lima et al., 2020). The incidence of clinical mastitis is in most flocks below 5% per year (Bergonier et al., 2003), but large differences between flocks are seen (Waage and Vatn, 2008; Grant et al., 2016). Most cases of clinical mastitis occur early in lactation (Waage and Vatn, 2008) and are caused by *Staphylococcus aureus* and *Mannheimia haemolytica* (Bergonier et al., 2003; Contreras et al., 2007; Gelasakis et al., 2015). Subclinical mastitis is common in sheep, with NAS being the most cultured bacterial species, with herd-level prevalence varying between about 25% and more than 90% (Bergonier et al., 2003; Gelasakis et al., 2015; Dore et al., 2016). *Staphylococcus aureus* and *M. haemolytica*, however, have also been cultured from clinically healthy udders. Intramammary infection with these agents can increase the SCC, and high SCC has previously been associated with reduced lamb growth, likely related to the reduced milk yield in these udders (Huntley et al., 2012). Intramammary infections have been suggested to transmit during lactation through the suckling of lambs. Specifically for *M. haemolytica*, it has been shown that lambs carry the bacteria in their mouths, thereby acting as vectors when cross-suckling between infected and noninfected animals (Mavrogianni et al., 2007; Gougoulis et al., 2008). Also for *Staph. aureus*, suckling lambs are likely involved in transmission, analogous to theories about transmission of IMI in cows via the milking machine (Zadoks et al., 2002). As IMI are likely to be the major reservoir for pathogens responsible for clinical mastitis, controlling the transmission of IMI may also reduce the incidence of clinical mastitis. However, transmission

and the species-specific dynamics of IMI in meat sheep have been poorly studied.

In this study, we investigate the dynamics of IMI in 15 meat sheep flocks in the first 6 wk of lactation. The aim was to (1) describe the bacterial species causing IMI in meat sheep and their associations with SCC, (2) estimate per species the proportion of chronic IMI and the incidence of new IMI in early lactation, and (3) compare the dynamics of IMI between flocks.

MATERIALS AND METHODS

Selection of Flocks and Ewes

Sheep farmers were invited to participate in this research through a call in the digital newsletter of the Dutch Sheep and Goat Farmers Organisation (NSFO), to all the members of the organization, which are mostly Texel breed farmers. In total, 15 flocks across the country were enrolled, and each flock was visited twice between February and April 2015. These farms were similar in management and husbandry. Around lambing, the sheep were kept in deep straw barns and were generally kept indoors during the first 2 to 4 wk after lambing. The sheep received roughage (mostly grass silage) with concentrates during this period. No antibiotics had been used at the dry-off, as drying off with antibiotics is very uncommon in Dutch sheep farms. Twelve farms only had Texel sheep, one farm had Texel and Flemish sheep, one had Texel and Swifter, and one farm only had Swifter sheep. The number of ewes >1 yr old varied between 15 and 177 per farm (average 82). The first visit took place during the lambing period or briefly after the end of the lambing period. For this visit, we aimed to enroll 15 ewes that had lambed within the previous 3 wk. The ewes were selected by the farmer, aiming to enroll 5 first-parity, 5 second-parity, and 5 older animals. The second visit was scheduled 3 wk after the first, and the same 15 animals were sampled again.

Collection and Analyses of Milk Samples

On each sampling occasion, all enrolled ewes were separated from their lambs for 2 h before sampling to prevent the lambs from suckling. Two squirts of milk were discarded, and then 2 milk samples were collected from each udder half. First, one sample was taken for SCC measurement (approximately 10 mL in a tube containing 0.05 mL of 5% sodium azide as preservative). Then the teat end was disinfected with cotton wool balls soaked in 70% alcohol, and the sample for bacteriology was collected (approximately 2 mL). The samples for SCC measurement were transported at room temperature and refrigerated at <6°C for a maximum of 3 d before analyses using a Combiscope 600 (Delta Instruments), calibrated with a cow milk standard. The samples for bacteriological culture were cooled with icepacks, shipped to the laboratory and cultured within 12 h after collection of the sample. The culturing was done in accordance with National Mastitis Council guidelines (Hogan et al., 1999) with some modifications: 2 milk samples of one ewe were streaked a blood agar plate, each sample on one half of the plate, and incubated at 37°C with 5% CO_2 for 24 to 48 h. Plates were inspected at 24 and 48 h. To increase sensitivity of the culture, a pre-enrichment step was used by incubating the original milk sample together with the plates. If no growth was found after 24 h, 10 μL of this incubated milk was streaked on a blood agar plate. The result of the bacteriological examination was considered culture-negative if no growth was seen after 48 h and <5 colonies grew from the enrichment. If ≥3 morphologically different colony types were found after 48 h culturing or from the enriched milk sample, but the culture at 24 h was negative, the sample was also considered culture negative. If the primary culture yielded ≥3 morphologically different colony types after 24 h, the sample was considered contaminated. If growth was found on the plates after 48 h, the pre-enrichment culture was ignored. If 1 or 2 colony types were found in the primary culture at 24 or 48 h or in the pre-enrichment if the primary culture was negative, these were subjected to species determination using MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics), as previously described by Bradley et al. (2015). Isolates with a MALDI-TOF identification score of >2.0 were considered identified at the species level. Scores between 1.7 and 2.0 were identified at the genus level. In the case of *Staphylococcus* species, these isolates were assumed to be NAS, as *Staph. aureus* is normally identified with high scores. Any colonies that had the appearance of a hemolytic coccoid bacteria, even if <5 colonies from the enriched culture or in a culture where ≥3 morphologically different colony types were seen, were streaked to obtain a pure culture and then subjected to MALDI-TOF identification.

Statistical Analysis

All records of ewes that had a missing record for either the first or the second sampling occasion were excluded from the data set. The SCC values were $\log_{10}$-transformed, and average and standard deviation (SD) $\log_{10}$SCC were calculated per culture result. The effect of IMI on $\log_{10}$SCC was determined using a linear regression model, with sampling (first or second) and culture result (10 classes) as fixed effects and ewe within

flock as random effects. The models were run using the lmer function from the lme4 library (Bates et al., 2015) in R version 3.3.3 (https://www.r-project.org/).

To summarize the transitions of culture results of the same udder half between the 2 sampling moments, we calculated the proportions of chronic, cured, and new IMI, analyzing all NAS as a group. The proportion of chronic IMI was calculated as the number of udder halves still positive at the second sampling for the same species (or group of species, as all NAS were considered as one group) divided by the total number of udder halves positive for that species at the first sampling. The proportion of cured IMI for a certain (group of) species was calculated as the number of udder halves culture-negative at the second sampling divided by the number of udder halves positive for that species at the first sampling. The proportion of new IMI was calculated as the number of udder halves positive for a certain (group of) species at the second sampling that were culture-negative at the first sampling, divided by the total number of udder halves culture-negative at the first sampling.

RESULTS

In 15 flocks, 460 udder halves of 230 sheep were sampled twice within a 21-, 22- or 23-d interval. Ewes were between 1 and 31 d postpartum at the first sampling moment and between 21 and 53 d postpartum at the second. For 76 records, no SCC value was recorded, of which 26 were from udder halves that gave no milk.

Non-*aureus Staphylococcus* species were the most commonly isolated group of bacteria (Table 1), and consisted of 12 different species. A large number (93/253) of all NAS could not be determined with enough confidence at the species level by MALDI-TOF, but had scores between 1.7 and 2.0 in the MALDI-TOF analysis and thus were identified at the genus level (i.e., *Staphylococcus* spp.). The most commonly identified NAS were *Staphylococcus equorum* (n = 54), *Staphylococcus chromogenes* (n = 40), *Staphylococcus simulans* (n = 37), *Staphylococcus epidermidis* (n = 9), and *Staphylococcus haemolyticus* (n = 7). Other species were found <5 times in our samples. Table 1 shows the bacteriological culture results and the $\log_{10}$SCC associated with the bacterial species at the 2 sampling times. The category of "other" bacteria consisted mainly of species that could not be identified by MALDI-TOF (score <1.7, n = 47), and genera that seemed to be of minor importance, such as *Bacillus*, *Micrococcus*, and *Aerococcus* and some species that were found in very low numbers, such as *Trueperella pyogenes* (n = 2), *Rothia amarae* (n = 2) and *Rothia nasimurim*, *Pseudomonas* spp., *Pantoea agglomerans*, and *Kocuria rhizophila* (all n = 1). Associations between infection status and $\log_{10}$SCC are shown in Table 2, which showed that *Staph. aureus* had a small but significant effect on $\log_{10}$SCC, comparable to the effect of NAS. In contrast, *M. haemolytica* and *Escherichia coli* strongly increased $\log_{10}$SCC. *Streptococcus* spp. had a moderate effect on $\log_{10}$SCC.

Table 3 gives the proportion of udder halves that had a chronic IMI and the proportions of cured and new IMI during the 3-wk study interval. This shows that about 30 to 40% of the *Staphylococcus*-infected udder halves were still positive 3 wk later, and a similar fraction of cases had become culture-negative. The remaining udder halves yielded a different culture result at the second sampling. In contrast, 6 out of 7 udder halves (86%) that were infected with *M. haemolytica*

Table 1. Culture results and associated $\log_{10}$-transformed SCC ($\log_{10}$SCC) of 460 udder halves of 230 sheep in 15 Dutch flocks sampled between d 1 and 31 postpartum (first sampling) and between d 21 and 53 postpartum (second sampling)

	First sampling				Second sampling				All	
			$\log_{10}$SCC				$\log_{10}$SCC			
Culture result	N	%	Mean	SD	N	%	Mean	SD	N	%
NAS	133	29	5.26	0.69	120	26	5.41	0.71	253	28
Staphylococcus aureus	9	2	5.58	0.88	27	6	5.20	0.73	36	4
Streptococcus spp.	9	2	5.41	0.57	13	3	5.40	0.55	22	2
Corynebacterium spp.	2	0	4.61	0.00	18	4	4.92	0.57	20	2
Mannheimia haemolytica	7	2	5.99	0.72	10	2	6.09	0.45	17	2
Enterococcus spp.	4	1	4.68	0.39	4	1	6.02	0.88	8	1
Escherichia coli	3	1	5.92	0.69	1	0	6.62	NA[1]	4	0
Other	29	6	4.91	0.49	53	12	5.21	0.64	82	9
Mixed culture	43	9	5.37	0.64	39	8	4.96	0.64	82	9
Culture negative	208	45	4.85	0.51	162	35	4.92	0.45	370	40
No milk	13	3	NA		13	3	NA		26	3
Total	460	100	5.07	0.64	460	100	5.16	0.65	920	100

[1]NA = not applicable.

Table 2. Regression model of the association between sampling moment and bacteriological culture result and $\log_{10}$-transformed SCC ($\log_{10}$SCC)[1]

Item	Level	β coefficient	95% CI (β)
Intercept		4.83	4.70 to 4.95
Sampling moment	First	Referent	
	Second	0.08	0.02 to 0.15
Culture result	Culture negative	Referent	
	NAS	0.32	0.23 to 0.41
	Staphylococcus aureus	0.30	0.11 to 0.49
	Streptococcus spp.	0.57	0.33 to 0.81
	Corynebacterium spp.	0.00	−0.23 to 0.23
	Mannheimia haemolytica	0.99	0.67 to 1.30
	Enterococcus spp.	0.25	−0.15 to 0.64
	Escherichia coli	0.95	0.41 to 1.49
	Other	0.15	0.02 to 0.28
	Mixed culture	0.20	0.07 to 0.33

[1]Data on $\log_{10}$SCC were available for 856 milk samples collected from both udder halves of 228 ewes in 15 flocks, which were sampled twice in lactation, between d 1 and 31 postpartum (first sampling time), and between d 21 and 53 postpartum (second sampling time).

at the first sampling were still positive at the second sampling. During the 3-wk interval, 21% of the culture-negatives at the first sampling became NAS-infected at the second sampling, 7% acquired a new *Staph. aureus* IMI, and 6% a new IMI by *Corynebacterium* spp., but for other species, the number of new IMI was <1%. Figure 1 gives the proportion of positive culture results by flock, showing an increase in udder halves positive for *Staph. aureus* and *Corynebacterium* spp., but also showing that the number of new *Staph. aureus* IMI within a flock seemed to depend on the number of *Staph. aureus* IMI at the first sampling in that flock: in 6 out of 7 flocks where *Staph. aureus* was cultured at the first sampling, the proportion had increased 3 wk later. Conversely, in 7 out of 8 flocks where *Staph. aureus* was not found in the first sampling, *Staph. aureus* was still absent at the second sampling. *Corynebacterium* spp. were almost absent at the first sampling, but first appeared in 8 out of 15 flocks at the second sampling. Figure 2 shows the proportion of positive culture results for the 3 most prevalent *Staphylococcus* species and the other *Staphylococcus* species (including nonidentifiable) by flock. This figure shows that different NAS species vary in prevalence distribution across flocks, but none of these species showed clear trends in increasing or decreasing prevalence over time.

DISCUSSION

The bacteriological findings in our study were comparable to previous studies in suckler sheep, with NAS being the most prevalent group of bacteria. The percentage of culture-negative samples (40%) was relatively low compared with previous studies, which reported >70% culture-negative samples (Zadoks et al., 2014; Blagitz et al., 2014; Persson et al., 2017). We found SCC to be significantly associated with positive culture results of different bacterial species (Table 2), but this effect was surprisingly small. Few previous studies have measured the association between SCC and IMI in suckler ewes. Persson et al. (2017) found a strong effect of *Staph. aureus* and *M. haemolytica* on SCC in their study, stronger than that observed in the current study, and also reported other species to affect SCC more than in our study. A possible reason for this may be that we used pre-enrichment of the samples to increase sensitivity of the bacteriological examinations. Indeed, when excluding the results from pre-enrichment, the percentage of culture-negative samples would be about 20% higher (data not presented), approaching the range of previously published studies (Blagitz et al., 2014; Zadoks et al., 2014; Persson et al., 2017). Specifically, a substantial proportion of all *Staph. aureus*-positive samples was found positive in the pre-enrichment (16/36, 44%). These samples had a geometric mean SCC that was approximately one-third of that in samples that yielded *Staph. aureus* in the primary culture (111×10^3 cells/mL vs. 326×10^3 cells/mL), although this was still higher than the geometric mean SCC of culture-negative samples. It is unclear how a *Staph. aureus* culture result from pre-enrichment should be interpreted. A small number of colony-forming units in a milk sample may be indicative of contamination, for instance by *Staph. aureus* from the teat skin, but may also represent an actual, but possibly less severe, IMI or a low-level shedding IMI (Sears et al., 1990). Infection by *M. haemolytica* was associated with the strongest increase in SCC, in line with Persson et al. (2017), confirming the pathogenicity of this pathogen in sheep. In meat sheep, *Staph. aureus* and *M. haemolytica* are the most important agents responsible for clinical mastitis (Koop et al., 2010; Omaleki et al.,

Table 3. Cross-tabulation of the culturing result at the first versus the second sampling of 460 udder halves of 230 sheep in 15 Dutch flocks sampled between d 1 and 31 postpartum (first sampling) and between d 21 and 53 postpartum (second sampling)[1]

Culture result at first sampling	Culture result at second sampling												
	NAS	*Staph. aureus*	*Strep.* spp.	*M. haemolytica*	*Enterococcus* spp.	*E. coli*	*Corynebacterium* spp.	Other	Mixed culture	Culture negative	No milk	Total	Proportion chronic	Proportion cure
NAS	55	5	4	0	1	0	4	15	8	41	0	133	0.41	0.31
Staphylococcus aureus	1	3	0	0	0	0	0	0	0	4	1	9	0.33	0.44
Streptococcus spp.	2	0	4	0	0	0	0	0	1	2	0	9	0.44	0.22
Mannheimia haemolytica	0	0	0	6	0	0	0	0	0	1	0	7	0.86	0.14
Enterococcus spp.	0	0	0	0	3	0	0	0	0	1	0	4	0.75	0.25
Escherichia coli	0	0	0	0	0	1	1	0	0	1	0	3	0.33	0.33
Corynebacterium spp.	0	0	1	0	0	0	0	0	0	1	0	2	0.00	0.50
Other	4	3	2	1	0	0	1	5	2	10	1	29	0.17	0.34
Mixed culture	14	0	0	0	0	0	0	6	6	17	0	43	0.14	0.40
Culture negative	44	15	2	3	0	0	12	27	22	83	0	208	0.40[2]	NA[3]
No milk	0	1	0	0	0	0	0	0	0	1	11	13	0.85	0.08
Total	120	27	13	10	4	1	18	53	39	162	13	460	0.39	0.35
Proportion new infections	0.21	0.07	0.01	0.01	0.00	0.00	0.06	0.13				0.50		

[1]Per culture result is indicated what proportion of udder halves stayed chronically infected (or stayed culture negative), was cured, and the proportion of new infections since the first sampling.

[2]Proportion chronic is, in this case, the proportion chronic culture-negative (i.e., the proportion of samples culture-negative at first sampling that were still culture-negative at the second sampling).

[3]NA = not applicable.

2011; Smith et al., 2015), but our study seems to indicate that *M. haemolytica* induces a much stronger SCC response than *Staph. aureus*, possibly attributable to immune evasion factors possessed by *Staph. aureus* that limit the primary immune response by the epithelial cells (Fu et al., 2013).

In addition to inducing the strongest SCC increase, *M. haemolytica* was also found to cause the most chronic infections in our study. Of all 7 *M. haemolytica*-positive udder halves at the first sampling, 6 (86%) were still positive 3 wk later. *Staphylococcus* and *Streptococcus* species caused much less stable infections; less than 45% of the positive udder halves at the first sampling were still positive for these species after 3 wk. This is in contrast with what has been found in dairy goats, where *Staphylococcus* species caused highly chronic IMI (Contreras et al., 1997; Koop et al., 2012). However, in line with what was mentioned above, the sensitive culturing protocol used in our study may have led to underestimation of the persistence, assuming that the relatively high number of positive cultures is partly the result of misclassification and thus does not represent all true IMI, which obviously cannot be persistent. Indeed, all 3 udder halves that were culture-positive for *Staph. aureus* at the first as well as the second sampling were found positive in the primary culture and not after pre-enrichment, whereas none of the samples that were positive after pre-enrichment at the first sampling were positive again for *Staph. aureus* at the second sampling moment. Overall, our study had a limited sample size, and the numbers of samples positive for a particular species were relatively small. Therefore, these results should be interpreted with caution.

We found a large proportion (37%) of samples to be unidentifiable at the species level but that were identified as *Staphylococcus* at the genus level. It is unclear why this was the case. Possibly, some rare *Staphylococcus* species were present in many of the sheep that were not present in the library used, although it seems unlikely that Dutch sheep harbor species that are uncommon elsewhere in the world. Another possibility is that it was caused by technical issues, such as suboptimal application of the colonies onto the target, although this was done by students under supervision of an experienced technician. Overall, we cannot be sure why so many isolates were inconclusively identified.

The number of udder halves positive for *Staph. aureus* and *Corynebacterium* spp. strongly increased during the 3 wk between the first and the second sampling (Figure 1), in contrast to NAS, which showed no clear increasing or decreasing trend (Figure 2). To our knowledge, no published studies have investigated IMI in meat sheep longitudinally during lactation. The transmission dynamics of IMI in suckler sheep may well

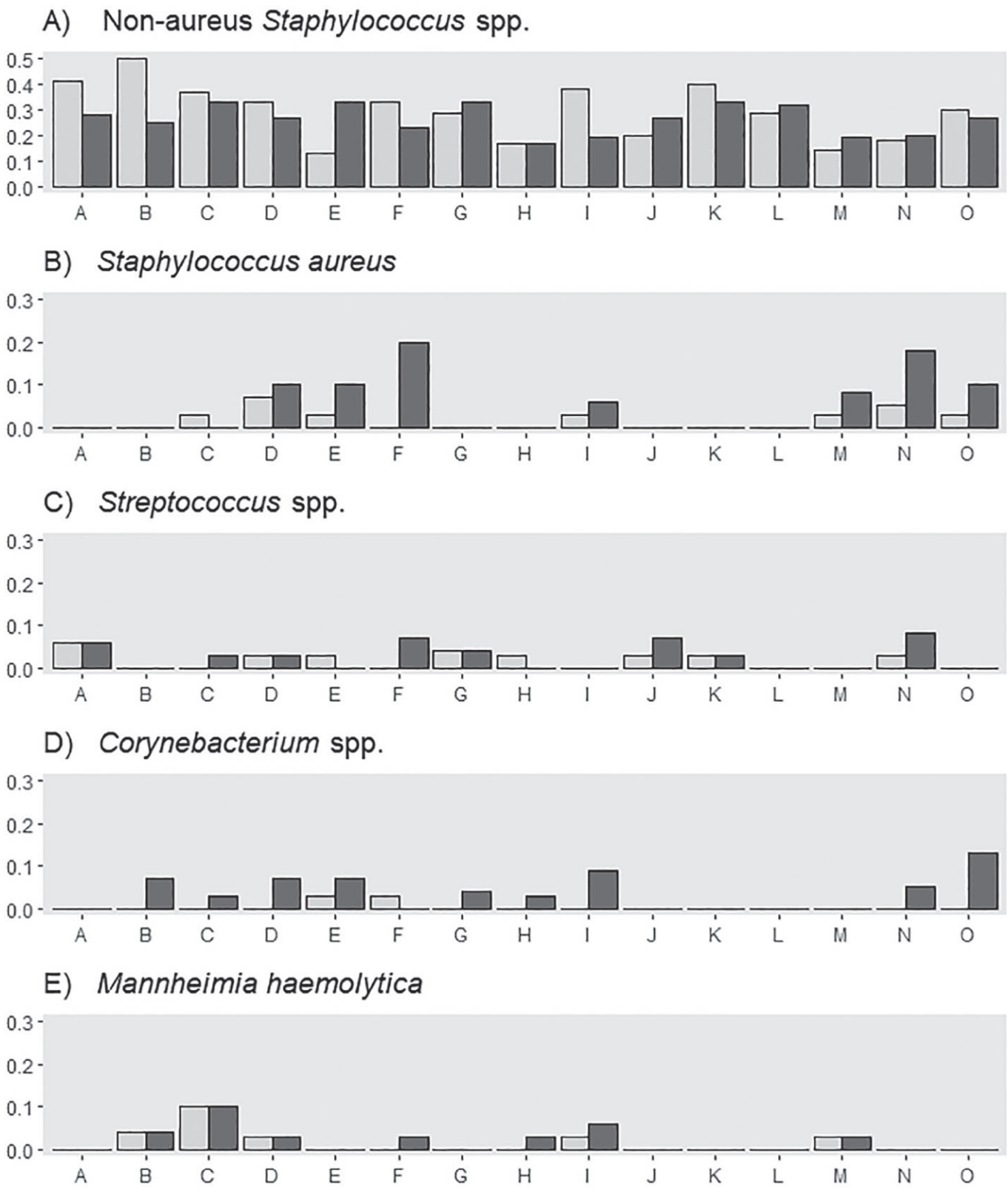

Figure 1. Within-flock proportion of udder halves culture-positive for (A) NAS, (B) *Staphylococcus aureus*, (C) *Streptococcus* spp., (D) *Corynebacterium* spp., and (E) *Mannheimia haemolytica*, based on milk samples collected from 460 udder halves of 230 sheep in 15 Dutch flocks (A through O) sampled between d 1 and 31 postpartum (first sampling, light gray bars) or between d 21 and 53 postpartum (second sampling, dark gray bars).

differ from that in dairy ewes, because the former are not milked and thus the milking machine or the (hand) milker cannot contribute to transmission. Therefore, pathogens are primarily transmitted by the lambs or by the environment. It has been shown that suckling by lambs predisposes to entrance of *Staphylococcus* spp. but also of *M. haemolytica* into the teat (Gougoulis et al., 2008). Figure 1 suggests that the number of new *Staph. aureus* infections was related to the number of infected animals at the previous sampling, indicative of contagious transmission between ewes. Therefore, controlling transmission of *Staph. aureus* early in lactation may help to control mastitis later in the lactation. The prevalence of *M. haemolytica*, in contrast, was stable between the 2 samplings. Possibly, the transmission of this pathogen is limited early in lactation and increases only later in lactation. It has been shown that lambs are the likely vectors of *M. haemolytica* because they carry the pathogen in their nasopharynx and are capable of infecting the udder during suckling (Gougoulis et al., 2008; Omaleki et al., 2015). If lambs are responsible for the transmission of mastitis pathogens, it is dif-

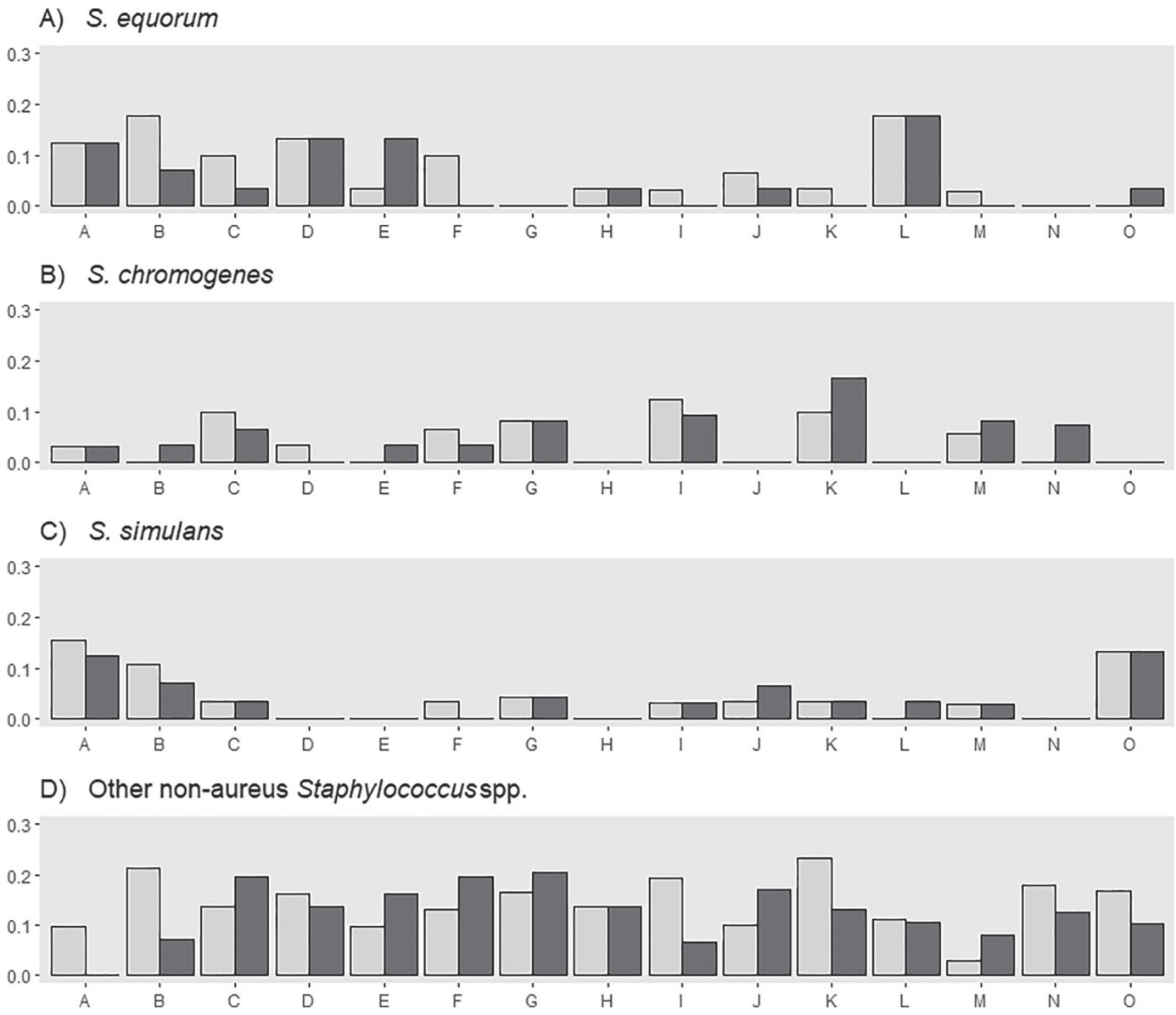

Figure 2. Within-flock proportion of udder halves culture-positive for (A) *Staphylococcus equorum*, (B) *Staphylococcus chromogenes*, (C) *Staphylococcus simulans*, and (D) other NAS based on milk samples collected from 460 udder halves of 230 sheep in 15 Dutch flocks (A through O) sampled between d 1 and 31 postpartum (first sampling, light gray bars) or between d 21 and 53 postpartum (second sampling, dark gray bars).

ficult to understand why we saw a strong increase in the prevalence of *Staph. aureus* and *Corynebacterium* spp., but not of *M. haemolytica*. Possibly, in early lactation there is still little cross-suckling by the lambs, limiting the likelihood of actual transmission between ewes; however, because suckling increases the chances for bacteria to enter the teat canal (Gougoulis et al., 2008), the proportion of IMI caused by bacteria residing on teat skin or the nasal cavity of the lamb (such as *Staphylococcus* spp. including *Staph. aureus*) may increase. That would imply that the increased *Staph. aureus* prevalence is actually not the result of between-ewe transmission, but within-ewe translocation of bacteria from skin to the mammary gland or transmission between lamb and ewe. Molecular typing of isolates from teat or udder skin and from the oral and nasal cavities of the lambs may establish the potential role of these sites as reservoirs for IMI. Future studies should follow ewes and their lambs longitudinally during the lactation to identify when the different pathogens transmit most and to identify if the lambs indeed are the primary vehicle of transmission. Specifically, such studies should include the period when ewes and their lambs are on pasture, because pasturing may affect the potential of the lambs to cross-suckle and the lower amount of concentrates and possibly minerals given on pasture may affect the overall health and immunity of the sheep. This information may be used to identify interventions to effectively limit transmission. Examples of such interventions may be the separation of animals suspected of having IMI, which would require a testing protocol, or interventions that would limit the amount of cross-suckling, such as creating smaller group sizes or applying feed interventions to stimulate milk yield and thus reduce the incentive for lambs to cross-suckle. Such interventions may ultimately contribute to mastitis control in suckler ewes.

CONCLUSIONS

Staphylococcus spp. are the most prevalent intramammary pathogens in meat sheep in early lactation, but they have a smaller effect on SCC and cause fewer chronic infections than *M. haemolytica*. Transmission of *Staph. aureus* seems to be substantial during early lactation; therefore, further research should be done to identify the drivers and routes of this transmission using longitudinal studies, in which also the dynamics of *M. haemolytica* in later lactation can be studied.

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