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Short communication

Nasal carriage of *Staphylococcus aureus* in dairy sheep

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Abstract:

The purpose of this study was to assess the prevalence of *Staphylococcus aureus* nasal carriage of dairy sheep in farms producing cheeses manufactured with raw ewe's milk.

Moreover, we investigated the genetic diversity of the 136 isolates recovered from the anterior nares of the ewes, from the ambient air of the milking parlor and from cheeses. The isolates were typed using pulsed-field gel electrophoresis (PFGE) of DNA *Sma*I digests.

The average nasal carriage in the flock was 29% of the nares of the ewes contaminated by *Staphylococcus aureus*. The genotyping results showed that strains with dominant pattern (named OV) and a genetically related isolates (named OV') by a majority (69.8%) are recovered from nasal carriage in dairy sheep in this area of France.

Key-words: *Staphylococcus aureus*; Pulsed-Field Gel Electrophoresis (PFGE); Ewe's nares; Genotyping; Epidemiology

Understanding the epidemiology and the ecology of *Staphylococcus aureus* (*S. aureus*) [reservoirs, transmission pathways, and risk factors] has resulted in excellent control of this major mastitis pathogen in many cow herds. Milking time hygiene measures that decreased cow to cow transfer were largely responsible for decreasing new *S. aureus* intramammary infections. The most common transmission pathway consist of transfer from an infected mammary gland to an uninfected gland via fomites, such as milking equipment, common udder cloths, or the milker's hands. However, milking time hygiene alone was insufficient in controlling the disease. The addition of dry-cow therapy, and especially, culling the chronically infected were needed to achieve low level of *S. aureus* intramammary infections. If there is little doubt that the primary and most important reservoir of *S. aureus* is the infected mammary gland, what about the others sources such the nares beside other sources as teat skin, udder skin, lips, eyes, vagina, rectum, sacral region, etc. It would suggest that total eradication of *S. aureus* in a flock is currently impossible (Roberson et al., 1994; Roberson et al., 1998). In

Human disease, *S. aureus* nasal carriage has been extensively studied in patients and healthy individuals. Cross-sectional surveys of *S. aureus* nasal carriage have designated individuals as either carriers or noncarriers (Kluytmans et al., 1997).

Some authors have used the pulsed-field gel electrophoresis to genotype *S. aureus* colonising human nasal carriers (Hu et al., 1995). Less information is available about dairy sheep, even though it is likely that the ecology of *S. aureus* in a dairy sheep farm is probably the same than in a bovine or goat dairy farm (Vautor et al., 2003). In this study, we focus on one of a probable *S. aureus* reservoir of contamination of the udder represented by the nares of the ewes producing the milk for the manufacturing of the ewe's farm cheeses. We used the pulsed-field gel electrophoresis (PFGE) to investigate the genetic diversity of the nares' *S. aureus* isolates and to understand genetic relatedness between isolates recovered from different farms.

Between May 2003 and February 2004, in the south-east of France, ten farms (named A to J) were enrolled in the study. The farms were located at a distance of 15-200 km from each other and had no epidemiological links between them. The flock size varied from 30 to 230 ewes of French milking sheep breeds. General husbandry methods applied in these flocks were different, particularly regarding feeding,

housing, or the presence of other animals on the farm, etc. Farms A-G had a mechanical milking procedure and farms H-J had a hand milking procedure. The average number of milking ewes sampled in each flock was 37. Thus, a total of 368 ewes were sampled: 227 in farms A to G and 141 in farms H, I, J. Ewes included in the study were sampled only once. Both the left and right anterior nares were swabbed by rubbing the dry cotton-wool swab inside of each nostril while applying an even pressure and rotating the swab. Within 2-5 hours the swabs were transported at 4°C and streaked directly on Baird-Parker rabbit plasma fibrinogen agar (BPRPFA) medium (BioMérieux, Marcy-l'Etoile, France), incubated for 12-48 h at 37°C. Ambient air was sampled by using three plates (90mm in diameter) with BPRPFA medium exposed to the environment of the milking room for 15 min and incubated for 12-48 h at 37°C. These plates were exposed, for all the farm, at 51 cm from above the ground of the milking room (at the udder level). Samples of cheese were plated on BPRPFA medium and incubated for 24-48 h as described by De Buyser (De Buyser et al., 1998). Confirmation of the identity of some isolates was performed, after demonstration of catalase positivity, by using the API STAPH system and Slidex®Stap Plus-Kit (BioMérieux, Marcy-l'Etoile, France). For the ewes' nares, only one colony was chosen for further analysis. Such procedures have been done, assuming that all morphologically similar colonies on a plate are formed by the

same strain. For the air and cheese, up to five colonies were taken into account.

A macrorestriction analysis of the chromosomal DNA of the cultures was performed with the restriction enzyme *Sma*I and subsequent pulsed-field gel electrophoresis. This genotyping system was chosen due to its high discriminatory power and excellent reproducibility (Tenover et al., 1994; Olive and Bean, 1999). A contour clamped homogeneous electrophoresis technique (CHEF) on the GenePath™ system (BIO-RAD Laboratories, California, USA) was used according to the instruction manual of the GenePath Group 1 reagent kit (BIO-RAD). Briefly, isolates of *S. aureus* were transferred to a BHI broth and incubated overnight at 37°C in a shaking incubator. Bacterial cells were washed and resuspended in the cell suspension buffer (provided with the kit). The suspension was mixed with an equal volume of 1% chromosomal-grade agarose to make plugs. Bacterial cells were lysed in the resuspension buffer containing lysozyme (25 mg/ml) and lysostaphin (2 mg/ml) after an incubation for 1 h at 37°C. Then, 500 µl of proteinase K buffer and 20 µl of proteinase K (600 U/ml) were added and the plugs were incubated for 16-20 h at 50°C. The restriction enzyme *Sma*I (5 U/µl) and its buffer were added and incubated for 16-20 h at 25°C. Digests of DNA were separated during 20 h by PFGE using 1% gel at the following

conditions: 14°C, 120° field angle, 6 V/cm, 5-35 s pulse times. On each gel, two control plugs with the reference strain of *S. aureus* (strain CIP 57.10, Institut Pasteur, Paris, France) were included. Gels were stained with ethidium bromide (1 mg/ml) and photographed under UV light.

Isolates of *S. aureus* were placed in groups of identical or related strains by comparing, using visual examination, the banding patterns produced. The scheme used by Tenover (Tenover et al., 1995) was employed to differentiate between indistinguishable (zero band differences), closely related (1-3 band differences), possibly related (4-6 band differences) and unrelated or different (> 6 band differences) strains.

Among a total of 368 ewes' nares, 29% were positive for *S. aureus*. The detailed results of the bacteriological examination for each farm is given in table 1. It is of interest to know the frequency of isolation, expressed as a percentage (number of positive *S. aureus* samples divided by total number of samples), because nasal carriage in dairy sheep may represent a major source of *S. aureus* for the contamination of milk's product, beside infected mammary gland. Although it could not be a direct contamination of the cheese it could serve as an important reservoir in the farm which could explain, despite strong hygienic procedures, the persistence of udder contamination. (Zecconi et al.,

2003). To our knowledge, no studies have been carried out in sheep dairy farms to assess the percentage of ewes' nasal carrier. In the general human population the authors agree in a mean carriage of 37.2% (13873 people) with variations according to population type (health care workers, patients hospitalised, drugs addicts, etc) and factors that may influence the rate of *S. aureus* nasal carriage (nasal abnormalities, age, hormonal status in women, ecology of nasal flora, viral infections of the upper respiratory tract, etc). In this general human population, the rate of *S. aureus* nasal carriage range from 19 to 55.1% (Kluytmans et al., 1997). Longitudinal studies, however, indicated that carriage patterns differ between individuals, and that 10 to 35% of individuals carry *S. aureus* persistently, 20 to 75% carry *S. aureus* intermittently, and 5 to 70% are persistently free of *S. aureus* (noncarriers) (Hu et al., 1995; Eriksen et al., 1995). The variation in reported rates results, at least partly, from differences in study populations, sampling and culture techniques, and criteria for the definition of persistent or intermittent carriage. To our knowledge, no studies have been carried out in dairy sheep, so the mean rate (29%) found in our field survey couldn't been compared with other authors works. To identify for each ewe the carriage patterns (persistent, intermittent, and noncarriage), as determined by some authors for human being (VandenBergh et al., 1999), we should have obtained nasal

ewe swab specimen during a 12-week follow-up period. Persistent nasal carriage is a unique characteristic of a fraction of the population, and the attribute “persistent” should be confined to those ewes for whom serial swab specimen cultures consistently yield *S. aureus*. The carrier state of each ewe could change over time. In human patients, the mean number of colony forming unit (CFU) of *S. aureus* that can be isolated from the anterior nares is higher in persistent carriers than in intermittent carriers, resulting in more extensive dispersal of staphylococci in the environment (White, 1961). In this field study, we found some ewes (11 ewes, data not shown) with high number of CFU (more than 100 CFU/23.7 mm²): these ewes are, perhaps, cases of persistent carriers. The others ewes (mean contamination with standard deviation 6.6+/-0.5 CFU/23.7 mm²) are intermittent carriers, probably contaminated by strains from ambient air. But, although the contamination rate of the ambient air had not been found high (only 5 farms/10 sampled were positive with *S. aureus*, at the height of 51cm from the floor), it is likely that the air in the milking room represents a *S. aureus* constant contamination for the nostrils of the ewe. That stresses a possible role of air as a vehicle for spread of *S. aureus*. To assess the contamination of ambient air, sampling should be performed at a height under 51 cm from the floor (Vautor et al., 2003) or by means of a Surface Air System pump (Albenzio et al., 2003).

The *S. aureus* PFGE typing results were presented in table 2 and 3. Some *S. aureus* PFGE patterns are illustrated in figure 1. PFGE is considered to be among the most reliable and reproducible whole-genome typing procedure (Tenover et al., 1994; Olive and Bean, 1999). Twenty one different patterns were found among isolates of *S. aureus* (table 3). One major pulsotype (named OV) was identified in 25 strains found in five farms: A, C, E, I and J. Four patterns were closely or possibly related to OV (same genetic lineage classified as related patterns), according to Tenover's criteria (Tenover et al., 1995), and were named OV'. The OV' patterns were found in farms B, D, E and G-J. The OV patterns were only absent in farm F. A previous study in these 10 dairy sheep farms, between October 2001 and April 2002 (Vautor et al., 2003), showed that a single clone of *S. aureus* is widely distributed both in infected mammary glands and in cheese produced from raw milk. In the present study, it is noteworthy when the *S. aureus* strains are recovered from the cheeses (farms B, D, F, G,.....), they are always isolated from the nares of ewes in the flock (except for farm I). That suggests that this organism, specially when this is the dominant *S. aureus* strain (OV or OV'), readily or intermittently colonizes nares and after contaminates the udder milk and subsequently the cheese. Our actual finding bring to the fore that ewe nasal carriage may represent a reservoir for *S. aureus* to contaminate other sites on the

sheep dairy farm. So, if the infected mammary gland is not the only source/reservoir of *S. aureus*, control measures should continue even in herds with low to non-existent levels of *S. aureus* intramammary infections.

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