STAPHYLOCOCCUS AUREUS NASAL CARRIAGE IN RABBITS

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ABSTRACT

Although nasal carriage has been described as a risk factor for *Staphylococcus aureus* infections in humans, little information exists in rabbits, where *S. aureus* is one of the most important pathogens responsible for a number of different types of infections. This study was designed to determine the extent of staphylococcal nasal carriage in Spanish farms with chronic staphylococcosis and to establish whether a relationship existed between nasal carriage and development of lesions. One hundred and sixteen rabbits with and without signs of staphylococcosis were obtained from six industrial rabbitries. Nasal swabs for microbiological assessments were obtained. Microbiological results showed that 56% of the animals carried *S. aureus* in their nasal cavities: 84.2% of the animals with staphylococcal-related lesions and 28.8% of the apparently healthy animals. Additionally, the *S. aureus* strains isolated from the nasal cavity and from the lesions, in 91.7% of animals, were clonally related. It is suggested that nasal carriage of *S. aureus* in rabbits can play an important role in the development of clinical infections.

Key words: *Staphylococcus aureus*, Rabbit, Nasal carriage, Risk factor, Lesions.

INTRODUCTION

Staphylococcus aureus is an adaptable, opportunistic pathogen with abilities to persist and multiply in a variety of environments and cause a wide spectrum of diseases in both humans and animals (Cucarella *et al.,* 2004). In humans, *S. aureus* is a major pathogen responsible for both nosocomial and community-acquired infections (Francois *et al.,* 2005), including skin and wound infections, toxic shock syndrome, arthritis, endocarditis, osteomyelitis, and food poisoning (Gao and Stewart, 2004; von Eiff *et al.,* 2001). In animals, staphylococcal infections cause substantial economic losses in livestock industry worldwide (Mork *et al.,* 2005). In rabbits this bacterium infects dermal lesions and invades subcutaneous tissues (Okerman *et al.,* 1984) causing different lesions including pododermatitis, multisystemic abscessation and mastitis (Segura *et al.,* 2007; Vancraeynest *et al.,* 2004). In humans, nearly one third of the population is currently colonized by *S. aureus* (Mainous *et al.,* 2006). Moreover, it has been reported that a substantial proportion of cases of *S. aureus* bacteraemia appear to be of endogenous origin, originating from colonization of the nasal mucosa (von Eiff *et al.,* 2001). However, in animals the exact importance of infection sources, such as the nares, is practically unknown due to the scarcity of reports evaluating this site of colonization. The aim of the present study was to establish the prevalence of nasal carriage of *S. aureus* in Spanish rabbitries with chronic staphylococcosis and to determine whether nasal strains are genetically related to strains obtained from staphylococcal-related lesions in the same individual rabbits.

MATERIALS AND METHODS

Farms and animals

A total of one hundred and sixteen rabbit does were studied. The animals came from six industrial rabbitries localized in the Spanish Mediterranean coast. These rabbitries had a clinical history of chronic staphylococcal infections. This resulted in the sampling of fifty-nine apparently healthy does (nares) and fifty-seven rabbit does with different types of gross lesions consistent with *S. aureus* infections (nares and lesions).

Pathological studies

The fifty-seven does with staphylococcal lesions were discarded by the owners. Rabbits were euthanized by an intravenous injection of barbiturate (Dolethal ®. Vétoquinol SA, Lure, France). A complete necropsy was performed and any gross lesions were recorded. Tissues were fixed in 10% neutral buffered formalin and dehydrated through graded alcohols before being embedded in paraffin wax. Several 4 μm thick sections were cut from each sample and stained by haematoxylin and eosin.

Bacteriological procedures

Both the left and right anterior nares were swabbed by rubbing a dry cotton-wool swab inside each nostril while applying an even pressure and rotating the swab. Standard microbiological studies were performed on both nasal swabs and different gross purulent lesions observed in the animals. Samples were inoculated on blood-agar (BioMérieux, Marcy l'Etoile, France) and they were incubated aerobically at 37ºC for 24-48 hours. *S. aureus* isolates obtained from nares and demonstrated lesions were included for further study. For each positive sample, several colonies were chosen for further analysis to evaluate the possibility of multiple strains in an animal.

Genotypic characterization of *S. aureus* strains

Staphylococcal chromosomal DNA was extracted using a Genelute Bacterial Genomic DNA Kit (Sigma) according to the manufacturer's protocol, except that the bacterial cells were lysed by lysostaphin (Sigma; 12.5 μg/ml) at 37ºC for 1 hour before DNA purification. Molecular typing, based on the analysis of the polymorphic regions of the *coa*, *spa* and *clfB* genes, was carried out as previously described (Viana *et al.*, 2007).

Statistical analysis

Differences in the occurrence of lesions between positive and negative nasal carriers were analysed using Fisher's exact test.

RESULTS AND DISCUSSION

The bacteriological analysis of the nasal swabs showed that 56% (65 out of 116) of the rabbits were nasal carriers of *S. aureus* (Table 1), a percentage higher than the percentage previously reported in humans where, in the general population, a mean carriage rate of 37.2% has been found (Kluytmans *et al.*, 1997). The 84.2% (48 out of 57) of the animals with lesions had *S. aureus* in their nostrils. This percentage is higher that previously reported in people with *S. aureus* skin lesions (65.9%) (Kluytmans *et al.*, 1997) and rabbits from Belgian flocks with chronic staphylococcosis (61.4%) (Hermans *et al.*, 1999). This high percentage of carriers in rabbits indicates that nasal carriage could be an important risk as a source of staphylococcal infection. The association between *S. aureus* nasal carriage and staphylococcal disease has been well documented in humans (Perl *et al.*, 2002; Williams, 1963) where higher nasal carriage rates were found in patients with *S. aureus* skin infections, compared to the general population (Kluytmans *et al.*, 1997).

Table 1: Positive and negative rabbits regarding nasal isolation of *S. aureus*, grouped by farm

	Farm 1			Farm 2			Farm 3			Farm 4			Farm 5			Farm 6			Total		
	n	+	-	n	+	-	n	+	-	n	+	-	n	+	-	n	+	-	n	+	-
Healthy	7	4	3	6	4	2	11	2	9	11	0	11	12	6	6	12	1	11	59	17	42
Lesions	6	6	0	8	8	0	11	7	4	8	5	3	12	10	2	12	12	0	57	48	9
Total	13	10	3	14	12	2	22	9	13	19	5	14	24	16	8	24	13	11	116	65	51

Healthy: Healthy animals; Lesions: Animals with clinical lesions in which *S. aureus* was isolated; n: number of studied animals; (+): number of *S. aureus* nasal carriers; (-): number of non-*S. aureus* nasal carriers

Nasal carriage of *S. aureus* is considered an important source of infection in humans (Perl *et al.*, 2002; Williams, 1963). In contrast, in animals there are few studies about the colonization of *S. aureus* in different body locations (Hermans *et al.*, 1999; Hermans *et al.*, 2000). To know the importance of the nasal carriage, 57 animals with different staphylococcal-associated lesions were analysed for the presence of *S. aureus* in the nares (Table 2), and compared with 59 apparently healthy animals. The 84.2% (48 out of 57) of rabbit does with lesions were nasal carriers in contrast to 28.8% (17 out of 59) of the apparently healthy rabbits (Table 1). It seemed that in rabbits with lesions the percentage of nasal carriers was higher than in apparently healthy animals ($P<0.0001$). However the individual analysis of the data from each farm indicated that only the farms 4 ($P=0.005$) and 6 ($P=0.00001$) had statistically significant differences and farm 3 approached statistical significance ($P=0.08$). No differences were observed in farms 1 ($P=0.192$), 2 ($P=0.165$) and 5 ($P=0.193$). Therefore a higher number of animals and farms must be studied to better establish this point.

A causal relation between *S. aureus* nasal carriage and infection has been previously described in other species (Perl *et al.*, 2002; Valentine and Hall-Smith, 1952; von Eiff *et al.*, 2001). This point has been supported by the fact that the nasal *S. aureus* strains and the intralesional strains shared the same genotype. In forty-eight animals existed the opportunity to compare nasal and lesional isolates. The *S. aureus* strains isolated from the nasal cavity and from the lesions in 91.7% (44 out of 48) of animals were clonally related. In the remaining four cases (F 1-5, F 2-3, F 2-6 and F 4-2) the strains were differ-rent (Table 2). This contradicts previous reports which suggested that staphylococci from the nose are different from the lesional staphylococci (Williams, 1963). In one animal (F 1-5) two genotypes were isolated from their nose; both different from the genotype isolated in the lesion (pododermatitis). It has been reported that more than one bio- and/or phage type can be circulating in rabbitries and be isolated even in the same rabbit (Hermans *et al.*, 1999). In our study it was not possible to know the chronological relation between the nasal carriage and the development of the lesions. However, it has been reported that nasal carriage of *S. aureus* plays a key role in the development of *S. aureus* infections (Kluytmans *et al.*, 1997). Different staphylococcal-associated lesions were observed (Table 2). Mastitis, pododermatitis and abscesses were the most frequently noted lesions. The mammary gland lesions were often macroscopically involving the glands. This type of infection was of a chronic and purulent character. In the palmar and plantar surfaces of the legs, moderate or advanced degrees of ulcerative pododermatitis were observed. Different sized abscesses, palpable from the outside or localized in internal organs were noted. In conclusion, nasal carriers could play a role in the pathogenesis of staphylococcal infections and can be a risk factor for the development of lesions (mastitis, abscess, pododermatitis, etc) though more studies are necessary to definitively affirm this assertion.

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Table 2: Relation between genotypes isolated from nares and lesions and the diagnosed pathology

Identification	Genotype from nares	Genotype from lesion	Pathology
F 1-1	A1/II1/δ	A1/II1/δ	Pododermatitis
F 1-2	A1/II1/δ	A1/II1/δ	Pododermatitis
F 1-3	A1/II1/δ	A1/II1/δ	Pododermatitis
F 1-4	B3/IV2/γ	B3/IV2/γ	Pododermatitis
F 1-5	**F3/II6/β and B3/IV1/γ**	**A1/II1/δ**	Pododermatitis
F 1-6	A1/II1/δ	A1/II1/δ	Abscess
F 2-1	A1/II1/δ	A1/II1/δ	Abscess
F 2-2	A1/II1/δ	A1/II1/δ	Vulvovaginitis
F 2-3	**A1/III7/δ**	**A3/III2/δ**	Mastitis/Pododermatitis
F 2-4	A1/II1/δ	A1/II1/δ	Pododermatitis
F 2-5	A1/II1/δ	A1/II1/δ	Mastitis
F 2-6	**A3/III2/δ**	**A1/II1/δ**	Mastitis/Abscess
F 2-7	A3/III2/δ	A3/III2/δ	Abscess
F 2-8	A3/III2/δ	A3/III2/δ	Abscess
F 3-1	A1/III1/δ	A1/III1/δ	Mastitis
F 3-2	A1/III1/δ	A1/III1/δ	Mastitis
F 3-3	A1/III1/δ	A1/III1/δ	Mastitis
F 3-4	A1/III1/δ	A1/III1/δ	Mastitis
F 3-5	A1/III1/δ	A1/III1/δ	Mastitis
F 3-6	A1/III1/δ	A1/III1/δ	Mastitis/Pododermatitis
F 3-7	A1/III1/δ	A1/III1/δ	Mastitis
F 3-8	**Negative**	A1/III1/δ	Mastitis
F 3-9	**Negative**	A1/III1/δ	Mastitis
F 3-10	**Negative**	A1/III1/δ	Mastitis
F 3-11	**Negative**	A1/III1/δ	Mastitis
F 4-1	A1/II1/δ	A1/II1/δ	Mastitis
F 4-2	**A1/II1/δ**	**A1/II1/κ**	Pustules
F 4-3	A1/II1/κ	A1/II1/κ	Pododermatitis
F 4-4	A1/II1/δ	A1/II1/δ	Pustules
F 4-5	A1/II1/δ	A1/II1/δ	Pododermatitis
F 4-6	**Negative**	A1/II1/δ	Pustules/Pododermatitis
F 4-7	**Negative**	A1/II1/δ	Mastitis
F 4-8	**Negative**	A1/II1/κ	Pododermatitis/Mastitis
F 5-1	**Negative**	A1/II1/δ	Mastitis
F 5-2	A1/II1/δ	A1/II1/δ	Mastitis
F 5-3	A1/II1/δ	A1/II1/δ	Mastitis/Pododermatitis
F 5-4	A1/II1/δ	A1/II1/δ	Mastitis
F 5-5	**Negative**	A1/II1/δ	Mastitis
F 5-6	A1/II1/δ	A1/II1/δ	Mastitis
F 5-7	A1/II1/δ	A1/II1/δ	Mastitis
F 5-8	A1/II1/δ	A1/II1/δ	Mastitis
F 5-9	A1/II1/δ	A1/II1/δ	Mastitis
F 5-10	A1/II1/δ	A1/II1/δ	Mastitis
F 5-11	A1/II1/δ	A1/II1/δ	Mastitis
F 5-12	A1/II1/δ	A1/II1/δ	Mastitis
F 6-1	A1/II1/δ	A1/II1/δ	Mastitis
F 6-2	A1/II1/δ	A1/II1/δ	Mastitis
F 6-3	A1/II1/δ	A1/II1/δ	Mastitis
F 6-4	A1/II1/δ	A1/II1/δ	Mastitis/Pododermatitis/Arthritis
F 6-5	A1/II1/δ	A1/II1/δ	Mastitis
F 6-6	A1/II1/δ	A1/II1/δ	Mastitis
F 6-7	A1/II1/δ	A1/II1/δ	Mastitis/Pododermatitis
F 6-8	A1/II1/δ	A1/II1/δ	Pododermatitis/Abscess
F 6-9	A1/II1/δ	A1/II1/δ	Mastitis
F 6-10	A1/II1/δ	A1/II1/δ	Mastitis
F 6-11	A1/II1/δ	A1/II1/δ	Pododermatitis
F 6-12	A1/II1/δ	A1/II1/δ	Mastitis

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