STAPHYLOCOCCUS AUREUS NASAL CARRIAGE COULD BE A RISK FOR DEVELOPMENT OF CLINICAL INFECTIONS IN RABBITS

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Abstract: Although nasal carriage has been described as a risk factor for Staphylococcus aureus infections in humans, there is a scarcity of studies about S. aureus nasal carriers in animals. In rabbits, 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 and to establish whether a relationship exists between nasal carriage and development of lesions. One hundred and sixteen rabbits with and without chronic signs of staphylococcosis from 6 industrial rabbitries were monitored. Nasal swabs for microbiological assessments were obtained from all animals. Microbiological results showed that 56% of the animals carried S. aureus in their nasal cavities with significantly higher incidence in animals with staphylococcal-related lesions (84.2%) compared to apparently healthy animals (28.8%). Additionally, the S. aureus strains isolated from the nasal cavity and lesions were clonally related in 91.7% of animals. This suggests that nasal carriage of S. aureus in rabbits could be a risk for development of clinical infections.

Key Words: Staphylococcus aureus, rabbit, nasal carriage, 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 (von Eiff et al., 2001; Gao and Stewart, 2004). In animals, staphylococcal infections cause substantial economic losses in livestock industry worldwide (Mork et al., 2005). In rabbits, this bacteria infects dermal lesions and invades subcutaneous tissues (Okerman et al., 1984) causing different lesions including pododermatitis, multisystemic abscessation and mastitis (Segura et al., 2007; Corpa et al., 2009). In humans, nearly one third of the population is currently colonised by S. aureus (Mainous et al., 2006). Moreover, it has been reported that a substantial proportion of cases of S. aureus bacteremia appear to be of endogenous origin, originating from colonisation of the nasal mucosa (von Eiff et al., 2001). The reported percentage of nasal carriers in different animal species is variable: 7.9% in horses (Burton et al., 2008), 29% in ewes (Vautor et al., 2005) and 32.1% in rabbits (Hermans et al., 1999). A longitudinal study in cattle observed variations in the rate of nasal carriers, increasing over time (Graveland et al., 2012).

The aim of the present study was to establish the prevalence of nasal carriage of S. aureus and to determine whether nasal strains are genetically related to strains obtained from staphylococcal-related lesions in the same individual rabbits.

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MATERIALS AND METHODS

Farms and animals

A total of 116 rabbit does were studied. The animals came from 6 industrial rabbitries localised in the Spanish Mediterranean coast. These rabbitries had a history of staphylococcal infections.

This resulted in the sampling of 59 apparently healthy does and fifty-seven rabbit does with different types of gross lesions consistent with \textit{S. aureus} infections (nares and lesions) (Table 1).

Pathological studies

The 57 does with staphylococcal lesions were culled by the owners. Rabbits were euthanised by an intravenous injection of barbiturate (Dolethal\textsuperscript{®}; 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

Standard microbiological studies were performed from both nasal swabs and different gross purulent lesions observed in the animals. 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. Samples were inoculated on blood-agar (BioMérieux, Marcy l’Etoile, France) and they were incubated aerobically at 37°C for 24-48 h. \textit{S. aureus} strains were identified on the basis of morphological growth characteristics and haemolytic properties (Devriese \textit{et al.}, 1996). For each positive isolate, several colonies were randomly selected and identified at the species level using molecular characterisation specific for \textit{S. aureus} as described below.

Genotypic characterisation of \textit{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 37°C for 1 h before DNA purification. Molecular typing, based on the analysis of the polymorphic regions of the \textit{coa}, \textit{spa} and \textit{clfB} genes, was carried out as previously described (Viana \textit{et al.}, 2007).

Statistical analysis

Differences in lesion incidence between positive and negative nasal carriers were analysed using Fisher’s exact test.

RESULTS

The bacteriological analysis of the nasal swabs showed that 56\% (65 out of 116) of the rabbits were nasal carriers of \textit{S. aureus}. Interestingly, 84.2\% (48 out of 57) of rabbits does with lesions were nasal carriers in contrast to 28.8\% of healthy rabbits does (Table 1).

<p>| Table 1: Positive and negative rabbits for nasal isolation of \textit{S. aureus} grouped by farms. |
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<tr>
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<td>Total</td>
<td>13</td>
<td>10</td>
<td>3</td>
<td>14</td>
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Healthy: Healthy animals; Lesions: Animals with clinical lesions in which \textit{S. aureus} was isolated; n: number of studied animals; (+): number of \textit{S. aureus} nasal carriers; (-): number of non-\textit{S. aureus} nasal carriers.
Staphylococcus aureus nasal carriers and lesions

(17 out of 59) of the apparently healthy rabbits (Table 1). Thus, the percentage of nasal carriers was higher in rabbits with lesions than in apparently healthy animals ($P<0.0001$).

To determine whether the nasally carried strains were genetically related with those strains isolated from lesions, isolates from 48 animals were typed, based on the analysis of the polymorphic regions of the coa, spa and clfB (Viana et al., 2007). These animals were selected because they had staphylococcal lesions and were simultaneously nasal carriers of S. aureus. The S. aureus strains isolated from the nasal cavity and from the lesions were clonally related in 91.7% (44 out of 48) of animals. In the remaining 4 cases the strains were different. The most frequently found genotype was A1/I1/δ.

Different staphylococcal-associated lesions were observed. Mastitis, pododermatitis and abscesses were the most frequently noted lesions. The mammary gland lesions were often macroscopically involving one or several 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 localised in internal organs, were noted. Finally, 1 animal had a vulvovaginitis, characterised by a purulent material in the vagina which also exuded from the vulva. In this case the uterine horns were not involved.

**DISCUSSION**

Bacteriological analysis of the nasal swabs showed that 56% of the rabbits were nasal carriers of S. aureus, a percentage higher than the percentage previously reported in rabbits (Hermans et al., 1999), which indicates that nasal carriage could be an important risk as a source of staphylococcal infection. In fact, nasal carriage was associated with the observation of clinical lesions by S. aureus, as 84.2% of rabbit does with lesions were nasal carriers in contrast to 28.8% of the apparently healthy animals. Similar findings have been described in humans, where S. aureus colonisation is associated with development of bloodstream or skin infection (Nassar and Ayus, 2001; van Belkum et al., 2009).

A causal relation between S. aureus nasal carriage and infection was further supported by the fact that the nasal S. aureus strains and the intralesional strains shared the same genotype, as previously described in other species (von Eiff et al., 2001; Perl et al., 2002). Using 3 polymorphic genes (coa, spa and clfB) and genotyping the S. aureus strains obtained from the 48 animals with lesions, it was shown that the S. aureus strains isolated from the nasal cavity were identical to that isolated from the lesions in 44 animals (91.7%). Feil et al. (2003) found no significant differences in the distribution of genotypes between strains isolated from carriers and those from patients with invasive diseases. In addition, a large study comparing S. aureus strains from healthy people vs. isolates that caused invasive disease failed to identify genetic markers associated with infection (Lindsay et al., 2006).

Nasal carriage of S. aureus is considered an important source of infection in humans (Perl et al., 2002; Marshall and McBryde, 2014). In contrast, in animals there are few studies on the colonisation of S. aureus in different body locations (Hermans et al., 2000). Studies carried out in cattle and sheep support the hypothesis that S. aureus genetic variations might also have implications for virulence capacity (Vautor et al., 2005; Stutz et al., 2011).

In conclusion, nasal carriers appear to play a key part in the pathogenesis of staphylococcal infections and could be a risk factor for the development of several lesions (mastitis, abscess, pododermatitis, etc). However, more studies are necessary to determine the main direction of colonisation (from nose to lesions or lesions to nose).

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**REFERENCES**


