World Rabbit Science

World Rabbit Sci. 2015, 23: 181-184
doi:10.4995/wrs.2015.3960

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

SELVA L., VIANA D., CORPA J.M.

Biomedical Research Institute (PASAPTA-Pathology group), Veterinary School, Universidad CEU Cardenal Herrera, Av. Seminario s/n, Moncada, 46113 Valencia, Spain.

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* bacteraemia 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.

Correspondence: L. Selva, lselva@uch.ceu.es. Received *June 2015* - Accepted *July 2015.*
http://dx.doi.org/10.4995/wrs.2015.3960

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 *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®; 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. *S. aureus* strains were identified on the basis of morphological growth characteristics and haemolytic properties (Devriese *et al.*, 1996). For each positive isolate, several colonies were randomly selected and identified at the species level using molecular characterisation specific for *S. aureus* as described below.

Genotypic characterisation 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 37°C for 1 h 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 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 *S. aureus*. Interestingly, 84.2% (48 out of 57) of rabbits does with lesions were nasal carriers in contrast to 28.8%

Table 1: Positive and negative rabbits for nasal isolation of *S. aureus* grouped by farms.

	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.

(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 *clf*B (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/II1/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 *clf*B) 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).

Acknowledgements: This study was supported by the Inter-ministerial Commission for Science and Technology (CICYT) of the Spanish Government (AGL2011-30170-C02-02 and AGL2014-53405-C2-2-P). The authors acknowledge Dr. E. A. Gómez for the statistical analysis and Prof. Sean (J.J) Callanan for revision of the manuscript.

REFERENCES

Burton S., Reid-Smith R., McClure J.T., Weese J.S. 2008. *Staphylococcus aureus* colonization in healthy horses in Atlantic Canada. *Can. Vet. J., 49: 797-799.*

Corpa J.M., Hermans K., Haesebrouck F.F. 2009. Main pathologies associated to *Staphylococcus aureus* infections in rabbits: a review. *World Rabbit Sci., 17: 115-125. doi:10.4995/wrs.2009.651*

Cucarella C., Tormo M.A., Úbeda C., Trotonda M.P., Monzón M., Peris C., Amorena B., Lasa I., Penadés J.R. 2004. Role of biofilm-associated protein bap in the pathogenesis of bovine *Staphylococcus aureus*. *Infect. Immun., 72: 2177-2185. doi:10.1128/IAI.72.4.2177-2185.2004*

Devriese L.A., Hendrickx W., Godard C., Okerman L., Haesebrouck F. 1996. A new pathogenic *Staphylococcus aureus* type in commercial rabbits. *Zentralbl. Veterinarmed. B, 43: 313-315. doi:10.1111/j.1439-0450.1996.tb00319.x*

Feil E.J., Cooper J.E., Grundmann H., Robinson D.A., Enright M.C., Berendt T., Peacock S.J., Smith J.M., Murphy M., Spratt B.G., Moore C.E., Day N.P. 2003. How clonal is *Staphylococcus aureus*?. *J. Bacteriol., 185: 3307-3316. doi:10.1128/JB.185.11.3307-3316.2003*

Francois P., Huyghe A., Charbonnier Y., Bento M., Herzig S., Topolski I., Fleury B., Lew D., Vaudaux P., Harbarth S., van Leeuwen, W., van Belkum A., Blanc D.S., Pittet D., Schrenzel J. 2005. Use of an automated multiple-locus, variable-number tandem repeat-based method for rapid and high-throughput genotyping of *Staphylococcus aureus* isolates. *J. Clin. Microbiol., 43: 3346-3355. doi:10.1128/JCM.43.7.3346-3355.2005*

Gao J., Stewart G.C. 2004. Regulatory elements of the *Staphylococcus aureus* protein A (Spa) promoter. *J. Bacteriol., 186: 3738-3748. doi:10.1128/JB.186.12.3738-3748.2004*

Graveland H., Wagenaar J.A., Verstappen K.M., Oosting-van Schothorst I., Heederik D.J.J., Bos M.E.H. 2012. Dynamics of MRSA carriage in veal calves: a longitudinal field study. *Prev. Vet. Med., 107: 180-186. doi:10.1016/j.prevetmed.2012.06.006*

Hermans K., De Herdt P., Devriese L.A., Hendrickx W., Godard C., Haesebrouck F. 1999. Colonization of rabbits with *Staphylococcus aureus* in flocks with and without chronic staphylococcosis. *Vet. Microbiol., 67: 37-46. doi:10.1016/S0378-1135(99)00028-0*

Hermans K., De Herdt P., Devriese L.A., Godard C., Haesebrouck F. 2000. Colonisation of rabbits with *Staphylococcus aureus* after experimental infection with high and low virulence strains. *Vet. Microbiol., 72: 277-284. doi:10.1016/S0378-1135(99)00179-0*

Lindsay J.A., Moore C.E., Day N.P., Peacock S.J., Witney A.A., Stabler R.A., Husain S.E., Butcher P.D., Hinds J. 2006. Microarrays reveal that each of the ten dominant lineages of *Staphylococcus aureus* has a unique combination of surface-associated and regulatory genes. *J. Bacteriol., 188: 669-676. doi:10.1128/JB.188.2.669-676.2006*

Mainous A.G., Hueston W.J., Everett C.J., Diaz V.A. 2006. Nasal carriage of *Staphylococcus aureus* and methicillin-resistant *S. aureus* in the United States, 2001-2002. *Ann. Fam. Med., 4: 132-137. doi:10.1370/afm.526*

Marshall C., McBryde E. 2014. The role of *Staphylococcus aureus* carriage in the pathogenesis of bloodstream infection. *BMC. Res. Notes, 7: 428. doi:10.1186/1756-0500-7-428*

Mørk T., Tollersrud T., Kvitle B., Jørgensen H.J., Waage S. 2005. Genetic diversity of *Staphylococcus aureus* isolated from ovine intramammary infections in Norway. *Vet. Microbiol., 106: 265-273. doi:10.1016/j.vetmic.2004.12.020*

Nassar G.M., Ayus J.C. 2001. Infectious complications of the hemodialysis access. *Kidney Int., 60: 1-13. doi:10.1046/j.1523-1755.2001.00765.x*

Okerman L., Devriese L.A., Maertens L., Okerman F., Godard C. 1984. Cutaneous staphylococcosis in rabbits. *Vet. Rec., 114: 313-315. doi:10.1136/vr.114.13.313*

Perl T.M., Cullen J.J., Wenzel R.P., Zimmerman M.B., Pfaller M.A., Sheppard D., Twombley J., French P.P., Herwaldt L.A. 2002. Intranasal mupirocin to prevent postoperative *Staphylococcus aureus* infections. *N. Engl. J. Med., 346: 1871-1877. doi:10.1056/NEJMoa003069*

Segura P., Martinez J., Peris B., Selva L., Viana D., Penades J.R., Corpa J.M. 2007. Staphylococcal infections in rabbit does on two industrial farms. *Vet. Rec., 160: 869-872. doi:10.1136/vr.160.25.869*

Stutz K., Stephan R., Tasara T. 2011. SpA, ClfA, and FnbA genetic variations lead to Staphaurex test-negative phenotypes in bovine mastitis S*taphylococcus aureus* isolates. *J. Clin. Microbiol., 49: 638-646. doi:10.1128/JCM.01148-10*

Vautor E., Abadie G., Guibert J.M., Chevalier N., Pepin M. 2005. Nasal carriage of *Staphylococcus aureus* in dairy sheep. *Vet. Microbiol., 106: 235-239. doi:10.1016/j.vetmic.2004.11.019*

van Belkum A., Melles D.C., Nouwen J., van Leeuwen W.B., van Wamel W., Vos M.C., Wertheim H.F., Verbrugh H.A. 2009. Co-evolutionary aspects of human colonisation and infection by *Staphylococcus aureus*. *Infect. Genet. Evol., 9: 32-47. doi:10.1016/j.meegid.2008.09.012*

Viana D., Selva L., Segura P., Penadés J.R., Corpa J.M. 2007. Genotypic characterization of *Staphylococcus aureus* strains isolated from rabbit lesions. *Vet. Microbiol., 121: 288-298. doi:10.1016/j.vetmic.2006.12.003*

von Eiff C., Becker K., Machka K., Stammer H., Peters G. 2001. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N. Engl. J. Med., 344: 11-16. doi:10.1056/NEJM200101043440102*