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COLONISATION OF RABBITS WITH *STAPHYLOCOCCUS AUREUS* AFTER EXPERIMENTAL INFECTION WITH HIGH AND LOW VIRULENCE STRAINS.

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ABSTRACT

Four groups of twelve rabbits each were inoculated in the nose with strains with suspected differences in virulence. In the two groups infected with strains from severe outbreaks, belonging to a rabbit-pathogenic biotype - phage type combination, six to twelve rabbits were found positive at the successive bacteriological samplings over a period of 28 days. In the two other groups, infected with strains obtained from rabbitries without a history of staphylococcosis, the number of *S. aureus* positive animals quickly became negative but increased again after one week to one to five positive animals until the end of the experiment. Two rabbits in each group inoculated with a high virulence strain developed purulent skin lesions while in the groups inoculated with low virulence strains, all animals remained clinically healthy. Results indicate that colonisation capacity is an important virulence determinant in rabbit staphylococcosis.

INTRODUCTION

In rabbits, staphylococcosis arises when *Staphylococcus aureus* infects small dermal lesions and invades subcutaneous tissue (Okerman et al., 1984). Clinical *S. aureus* infections in individual rabbits all have a similar appearance, with lesions of pododermatitis, subcutaneous abscesses and mastitis (Hagen, 1963; Okerman et al., 1984; Carolan, 1986; Holliman and Girvan, 1986; Devriese et al., 1996). Sporadically, internal organ abscesses are observed, predominantly in lungs, liver and uterus. This can lead to poor growth, infertility and death. Suckling young may die as a result of agalactia in the doe. In rabbit flocks, two types of *S. aureus* infections can be distinguished. In the first type, the infection remains limited to a small number of animals and is of minor economic importance. In the second, *S. aureus* infection results in epidemic spread of disease in the rabbitry. This leads to chronic problems and a subsequent decline in production. *S. aureus* virulence factors determining this epidemic spread of disease in rabbits, are not known.

A recent epidemiological investigation on the prevalence of *S. aureus* infections in industrial rabbitries demonstrated that in flocks experiencing chronic problems of staphylococcosis, rabbits were usually more intensely colonised with *S. aureus* compared to rabbitries without clinical signs of staphylococcosis. This might indicate that the ability of *S. aureus* strains to colonise contributes to their virulence for rabbits. Therefore in this study, the colonisation of rabbits with *S. aureus* following experimental infections with presumed high and low virulence strains was examined.

MATERIALS AND METHODS

Rabbits and animal facilities

Eight-week-old albino hybrid rabbits (Hycote, Ribecourt-La-Tour, France) were purchased from a commercial producer and used for experimental infections. Before the onset of the experiment,

samples were taken from the nares, the auditory canal, the interdigital skin, the medial side of the right foreleg, the axillar and inguinal skin region, the skin around the nipples, the perineum and the vagina or preputium of each experimental rabbit, and examined bacteriologically for the presence of *S. aureus*. All samples were negative. The animals were kept individually in wire-floored cages on three levels. Previous to the experiment, cages were thoroughly disinfected with denatured ethanol and fumigated with formaldehyde. The rabbits received food and water ad libitum.

***S. aureus* strains**

S. aureus strains used in these experiments were the isolates KH 15, KH 365, KH 103 and KH 171, which were all obtained from Belgian rabbitries (Hermans et al., accepted for publication). For the classification of these four *S. aureus* strains into biotypes, their beta-hemolysin- and staphylokinase-production and their growth type on crystal violet agar, were examined as described previously (Devriese, 1984). Phage typing was accomplished using bacteriophages of the international typing set for human *S. aureus* strains. Lytic reactions were examined at 100-fold Routine Test Dilution (Parker, 1962).

Strain KH 15 belonged to the phage type 42E/81 and to the human biotype, which is beta-hemolysin and staphylokinase positive and exhibits growth type C on crystal violet agar. Strain KH 365 represented phage type 52A/53/85/+ and biotype mixed CV-C, which is beta-hemolysin positive, staphylokinase negative and shows growth type C on crystal violet agar. Both isolates were obtained from two rabbitries that did not have a history of staphylococcosis-associated problems. For this reason, both strains were presumed to be low virulent, and are referred to as “low virulence” strains from this point on.

Strains KH 103 and KH 171 both belonged to the phage type 3A/3C/55/71 and the biotype mixed CV-C (beta-hemolysin-positive, staphylokinase negative and growth type C on crystal violet agar). Literature data suggest that strains of this biotype-phage type combination is highly virulent (Okerman et al., 1984, Carolan, 1986, Holliman and Girvan, 1986, Devriese et al., 1996). Strains KH 103 and KH 171 had been isolated from two different rabbitries suffering from severe problems of mastitis in breeding does and subcutaneous abscesses in broiler rabbits. In addition the rabbitry from which strain KH 171 was isolated was affected with staphylococcal pododermatitis in rabbits of all ages and pustular dermatitis in newborn rabbits. These two strains were presumed to be highly virulent, and are called “high virulence” strains from this moment on.

Strains were grown at 37 ° C on Columbia agar (Gibco, Paisley, Scotland) with 5 % ovine blood in a 5 % CO₂-enriched environment for 24 hours and checked for purity. The bacteria were harvested in sterile phosphate-buffered saline (PBS), centrifuged at 1700 x g at room temperature and resuspended in RPMI 1640 (Gibco, Paisley, Scotland) with 10 % fetal calf serum. The number of colony-forming units (CFU) of this suspension was determined by plating 10-fold dilutions on Columbia blood agar. The bacterial suspensions were stored overnight at 4 ° C, centrifuged at 1700 x g at room temperature, resuspended in sterile PBS and adjusted to a concentration of 2 x 10¹⁰ CFU ml⁻¹.

Experimental design

Infection groups 1, 2, 3 and 4, each consisting of twelve rabbits, were inoculated in both nostrils with 50 µl inoculum containing 1 x 10⁹ CFU of strains KH 15, KH 365, KH 103 or KH 171, respectively. Eight rabbits were inoculated with 50 µl of sterile PBS in each nostril and served as non-infected control animals. All four infection groups, each with two negative control rabbits, were kept in separate rooms. Direct contact between rabbits of the same infection group was not possible.

On days 1, 3, 5, 7, 9, 14, 21 and 28 after inoculation, swab samples were taken from the nares, the auditory canal, the interdigital skin, the medial side of the right foreleg, the axillar and inguinal skin region, the skin around the nipples, the perineum and the vagina or preputium of each rabbit and examined bacteriologically for the presence of *S. aureus*. Daily, rabbits were inspected for the occurrence of clinical signs and lesions.

Bacteriological examination

Bacteriological examination of all samples was performed on Columbia agar (Gibco, Paisley, Scotland) supplemented with 5 % ovine blood. All plates were incubated for 24 hours at 37 ° C in a 5 % CO₂ atmosphere. *S. aureus* colonies were identified on the basis of morphological growth characteristics, DNase activity and beta-haemolytic properties (Devriese et al., 1996).

The number of *S. aureus* colonies isolated from the different sampling sites was determined semiquantitatively. On this basis, four categories were distinguished: 0 (no *S. aureus* growth), 1 (1 to 10 colonies), 2 (11 to 50 colonies) and 3 (> 50 *S. aureus* colonies), and processed as such in statistical analysis. A rabbit was considered infected when at least one colony was isolated from at least one sampling site.

Seventeen *S. aureus* isolates that were isolated at regular intervals throughout the experiment, were biotyped (Devriese, 1984) and phage typed (Parker, 1962) to check the identity of the strain.

Statistical analysis

The number of positive sampling days for each rabbit from day 3 until the end of the experiment was compared between the four infection groups, using Kruskal-Wallis one way non-parametric analysis of variance. The Kruskal-Wallis test was also used for comparison of semiquantitative sampling results of the four infection groups, from day 3 until the end of the experiment. The significance level used was 0.05.

RESULTS

The uninfected control animals remained negative for *S. aureus* during the whole experiment.

The number of rabbits that were found positive for *S. aureus* at the successive samplings between day 1 and day 28 following inoculation with strains KH 15, KH 365, KH 103 and KH 171, is shown in Figure 1. On day 1, *S. aureus* was isolated from all twelve rabbits inoculated with strains KH 103, KH 171 and KH 365, and from eleven of the twelve rabbits inoculated with strain KH 15. After day 1, the *S. aureus* isolation rates were similar in the two groups of rabbits inoculated with the “low virulence” strains KH 15 and KH 365 and differed from those in rabbits inoculated with the “high virulence” strains KH 103 and KH 171. In the first two groups, the number of *S. aureus* positive animals quickly decreased to become zero at day 5 postinoculation. However, starting from day 7 and 9, *S. aureus* was again isolated in two animals of group 1 and in one animal of group 2, respectively. During the following samplings, in both groups one to five animals consistently yielded *S. aureus*. In groups 3 and 4, the number of *S. aureus* positive animals tended to decrease gradually. At the end of the experiment, six rabbits of group 3 and eight rabbits of group 4 were still colonised with *S. aureus*.

The mean scores of colony growth on the different bodysites demonstrated a more intense colonisation in the groups 3 and 4 than in the groups 1 and 2 (Figure 2).

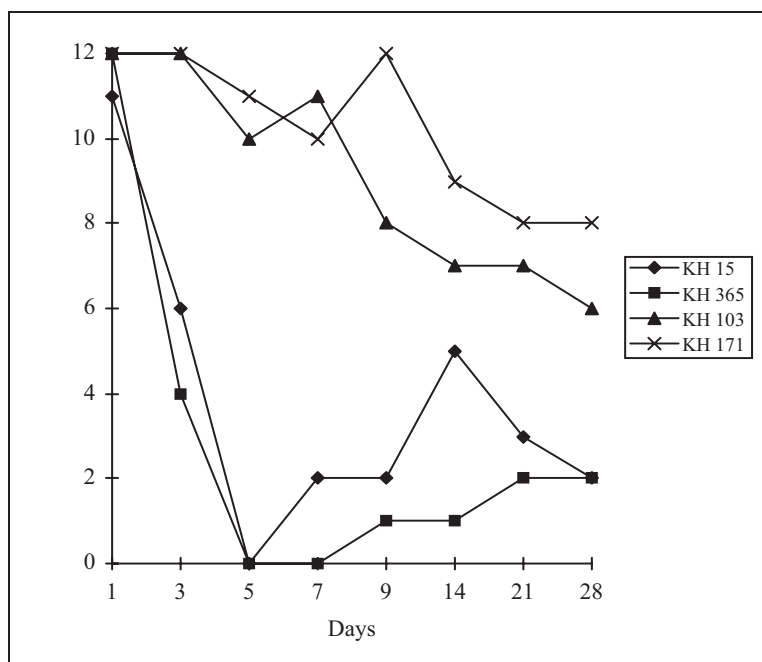


Figure 1: Number of rabbits found positive for the presence of *S. aureus* at the successive samplings following inoculation with strains KH 15, KH 365, KH 103 and KH 171.

Statistical analysis showed that the number of positive sampling days from day 3 until the end of the experiment, was significantly higher ($p < 0.02$) in rabbits of groups 3 and 4, both inoculated with “high virulence” *S. aureus* strains, compared to rabbits of groups 1 and 2, inoculated with “low virulence” strains. No significant differences were measured between group 1 and group 2, or between group 3 and group 4. Furthermore, statistical analysis of semiquantitative readings of colony growth indicated a more intense colonisation in the groups 3 and 4 than in the groups 1 and 2 ($p < 0.001$). Differences between group 1 and 2, and between group 3 and 4 were not significant.

After inoculation, *S. aureus* bacteria spread from the nose towards the other body sites sampled in all rabbits of the four infection groups. This spread was greater in rabbits inoculated with “high virulence” *S. aureus* strains than in rabbits inoculated with “low virulence” strains, as is shown in Figure 3.

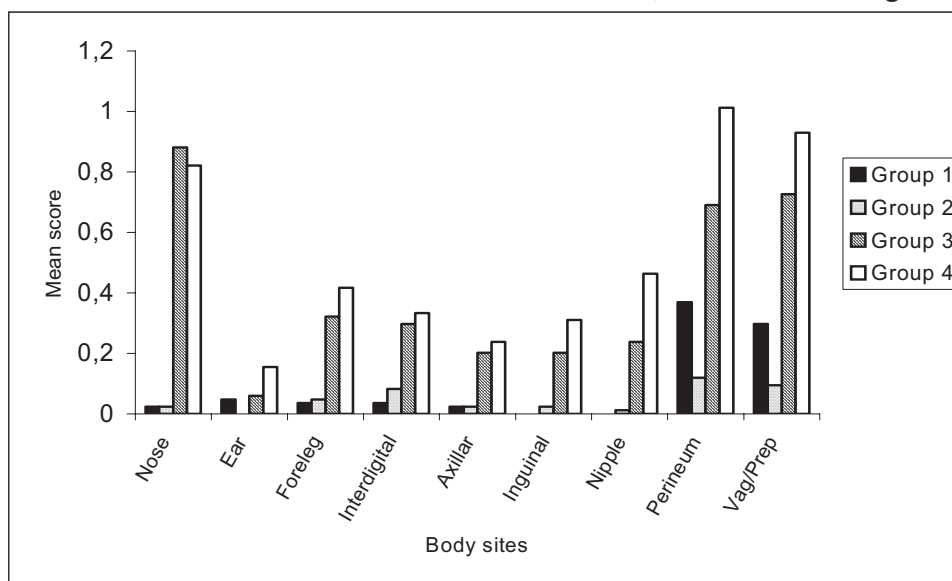


Figure 2: Mean semiquantitative scores of colony growth from day 3 until the end of the experiment, in nine different body sites of twelve rabbits per group experimentally inoculated with “low virulence” *S. aureus* strain KH 15 (Group 1), “low virulence” strain KH 365 (Group 2), “high virulence” strain KH 103 (Group 3) and “high virulence” strain KH 171 (Group 4).

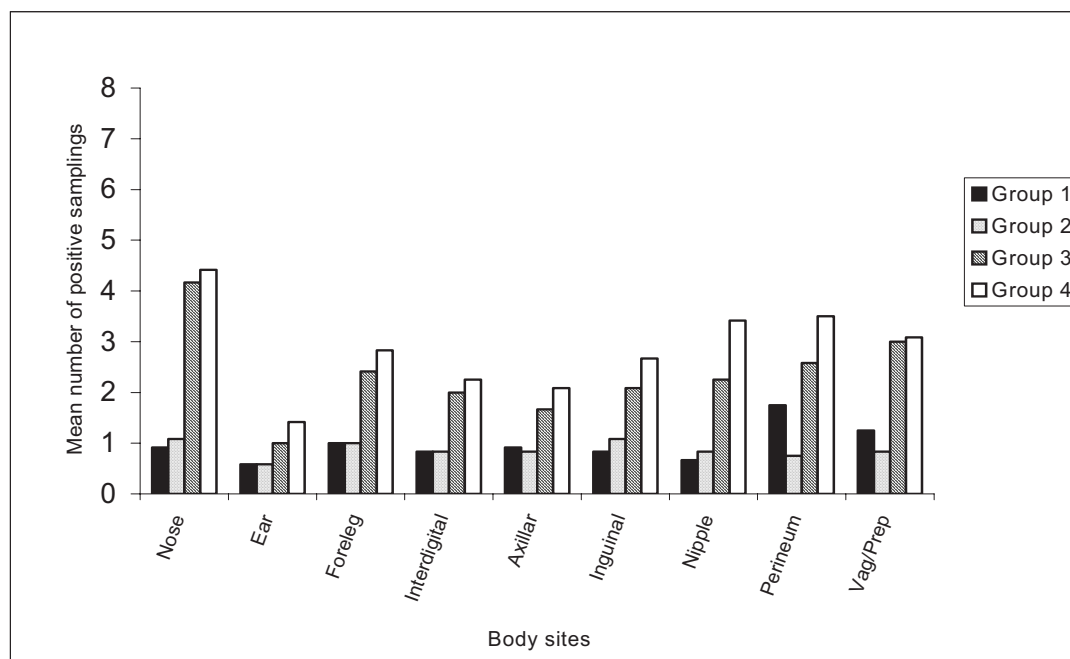


Figure 3: Mean number of positive samplings over eight sampling days between day 1 and the end of the experiment, in nine different body sites of twelve rabbits per group experimentally inoculated with “low virulence” *S. aureus* strain KH 15 (Group 1), “low virulence” strain KH 365 (Group 2), “high virulence” strain KH 103 (Group 3) and “high virulence” strain KH 171 (Group 4).

Strains that were isolated and retyped from the four experimental groups during the experiment all belonged to the same biotypes and phage types as used in the inoculum.

Clinically apparent lesions were observed in two rabbits inoculated with strain KH 103 and in two rabbits inoculated with strain KH 171. The animals showed small abscesses of the nose, the skin, the paws and the perineum. Lesions were first noticed between day 7 and day 14 postinoculation and lasted until the end of the experiment. From all these lesions, *S. aureus* was isolated. In rabbits inoculated with the “low virulence” strains and in the uninfected control animals, no lesions were found.

DISCUSSION

In this study, the colonisation capacity of “high virulence” and “low virulence” *S. aureus* strains was examined. One day postinoculation, *S. aureus* was isolated from 47 of the 48 inoculated rabbits. This does not necessarily indicate long-term colonisation of rabbits but may as well demonstrate that mechanical clearance mechanisms of the host (Reece, 1984), such as sneezing (Spörri, 1976), had not yet been able to remove the bacteria of the inoculum. The latter is supported by the fact that in both groups inoculated with the “low virulence” strains, the number of positive animals dramatically decreased during the following samplings.

For rabbits experimentally infected with the “low virulence” strains KH 15 and KH 365, the number of positive animals decreased quickly and became zero on day 5 postinoculation. In the groups inoculated with the “high virulence” strains, ten or eleven of the twelve rabbits remained positive during this period. Furthermore, the number of positive sampling days and the number of colonies isolated throughout the experiment, were significantly higher in rabbits inoculated with “high virulence” strains than in groups inoculated with “low virulence” strains. This indicates that the “high virulence” strains have a better ability to colonise the host, and that colonisation capacity may play a role in the virulence of *S. aureus* strains in rabbits.

When only sampling results after day one were taken into account, it could be seen that both “high virulence” strains colonised the nose in 22 of the 24 rabbits. The “low virulence” strains were only found in four out of 24 rabbits. This indicates that the ability of these “low virulence” strains to colonise the nose is low. However, it may be possible that these strains are capable of colonising other body sites.

Additionally, it was found that four rabbits inoculated with the “high virulence” strains KH 103 and KH 171, developed symptoms of staphylococcosis, while clinical signs or lesions were not found in rabbits that had been inoculated with “low virulence” *S. aureus* strains. This may reflect the situation in the field. Whether virulence of the strains is associated only with their colonisation capacity or also with other properties such as the ability to create or invade dermal lesions, needs further examination.

As a conclusion of field observations and results obtained in the present experimental study, it can be stated that the two groups of rabbit *S. aureus* strains used in this experiment are indeed of different virulence. The colonisation capacity should be regarded as an important virulence factor.

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