

PREVALENCE OF *STAPHYLOCOCCUS AUREUS* BIOTYPES IN COMMERCIAL RABBIT FARMS

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ABSTRACT

A cross sectional study was undertaken to estimate *Staphylococcus aureus* prevalence in rabbit flocks under rational production conditions. Fifteen farms were by convenience selected and 60 does were systematically sampled. Nose, ear, abdomen and interdigital skin swabs were collected from 900 does and data about flock production and flock health condition were recorded.

Results report a mean inside flock *S. aureus* prevalence of 77.6% (CI95% 66.4-88.2) of the selected sample. Three *S. aureus* biotypes were detected: the human one and two non host specific biotypes, mixed CV-C and mixed CV-A. Most of the flocks displayed *S. aureus* strains of just one biotype. However, in four flocks two biotypes were isolated: three flocks where one biotype was predominant and one unit where mixed CV-C and human biotypes were uniformly distributed. The among flocks distribution of *S. aureus* biotypes was correlated with the commercial source of breeders. Finally, statistic analysis of productive parameters related the presence of the mixed CV-C biotype to a lower number of weaned rabbits.

Key words: *Staphylococcus aureus*, Rabbit, Biotype.

INTRODUCTION

Concerns about acquired resistance to antimicrobials and the necessity to reduce production costs are leading towards a disease eradication policy by selecting rabbit breeders free from pathogens, such as high virulence *Staphylococcus aureus* strains (HV-SA).

HV-SA strains are well known (Devriese *et al.*, 1981) and, taking into account the rapid spreading of HV-SA infection within the flock and the subsequent production losses due to chronic staphylococcosis, Hermans (2003) has recently realised that culling of the entire infected rabbit flock is the only way to eradicate the disease.

Selection of HV-SA free breeders will increase their commercial value and optimise productive parameters. In order to apply an eradication policy it is necessary to acquire data about diffusion and impact of this specific microorganism and the resulting disease in rational rabbit production.

Some European breeding producers have actually implemented an eradication policy of HV-SA based on biotype CV-C as target, they are well aware that the biotype comprise both high virulence *S. aureus* strains (belonging to the 3A/3C/55/71 phagotype) and low virulence stains as well (Devriese *et al.*, 1981). Aim of our study was to estimate the within and between herd prevalence of *S. aureus* and its biotypes in rabbit reproduction units, moreover to estimate whether, in our production system, same HV-SA are associated with an increased spread of the within flock infection as previously described (Hermans *et al.*, 1999). Finally we investigate on the association of HV-SA as outcome variable of breeders commercial source and as risk factor for production losses.

Fifteen herds were enrolled and tested for *S. aureus* and its biotypes. The report gives preliminary results of a survey of HV-SA prevalence in Italian rabbit farms and will be completed with biomolecular testing of isolates.

MATERIALS AND METHODS

Fifteen flocks were sampled by convenience from the 567 rabbit intensive flocks of the Italian Veneto region. Within selected flocks 60 breeders were chosen for systematic sampling and enrolled for screening of *S. aureus* carriage. Collection of analytical samples from each rabbit and subsequent processing was described in a previous paper (Agnoletti *et al.*, 2007). One clone of *S. aureus* for each rabbit was processed as described by Devriese (1981) to define biotype. Each sampled rabbit was clinically examined to detect mastitis and abscesses; to quantify lesions severity a score was used (0 = no lesions; 1 = low severity lesions; 2 = medium severity lesions and 3 = high severity lesions).

Moreover a general anamnesis referred to staphylococcosis was registered; the latter variable mostly summarizes the overall owner recall of breeder replacement due to mastitis and abscesses. The productive parameters, mean number of born kits, mean number of weaned rabbits, percentage of breeder replacement and commercial source of breeders were collected as well. Student's exact test and linear regression were used to estimate associations between variables.

RESULTS AND DISCUSSION

S. aureus was isolated in all examined flocks; a within flock prevalence ranging from 41.6 up to 96.6% and a mean of 77.6% (CI95% 66.4–88.2) was calculated (Table 1). Hermans (1999) reported a 60.5% *S. aureus* mean prevalence in Belgian rabbit flocks (Hermans *et al.*, 1999).

Three biotypes were identified out of the 699 *S. aureus* strains collected: the human one and two non host specific biotypes: mixed CV-C and mixed CV-A. Eleven flocks hosted just one biotype; in four flocks two biotypes were isolated: three flocks where one biotype was predominant and one unit where mixed CV-C and human biotypes were uniformly distributed.

Between flocks different distribution of *S. aureus* biotypes can be expected to be related to commercial origin of breeders as some suppliers have adopted an eradication policy for *S. aureus* mixed CV-C biotype. When flocks are classified according to the presence of a certain biotype, the variable is associated with the commercial origin for both mixed CV-C biotype (P=0.002) and human biotype (P =0.028).

Table 1: Prevalence of *Staphylococcus aureus* and its biotypes

Farm	Anamnesis*	Breeders commercial source****	<i>S. aureus</i> within flock prevalence	<i>S. aureus</i> biotyping percentage (%)				<i>S. aureus</i> biotypes*** within flock prevalence	
				Mixed CV-C (%)	Human (%)	Mixed CV-A (%)	NT (%)**	Mixed CV-C (%)	Human (%)
1	0	a	93.3	0	100	0	0	0	93.3
2	3	d	95	40.3	50.9	0	8.8	38.3	48.3
3	3	b	53.3	100	0	0	0	53.3	0
4	0	a	90	0	98.1	0	1.9	0	88.3
5	0	a	83.3	0	98	0	2	0	81.7
6	0	b	83.3	100	0	0	0	83.3	0
7	0	a	93.3	0	98.2	0	1.8	0	91.7
8	1	b	91.6	78.2	0	0	21.8	71.7	0
9	1	b	85	78.4	9.8	0	11.8	66.7	8.3
10	2	a	80	100	0	0	0	80	0
11	0	b	46.7	100	0	0	0	46.7	0
12	3	c	48.3	100	0	0	0	48.3	0
13	3	b	83.3	94	0	6	0	78.3	0
14	2	b	96.7	96	4	0	0	93.3	0
15	0	a	41.6	0	100	0	0	0	41.7
mean			77.6	46.2	30.8			43.9	30.2

*0: no staphylococcosis reported; 1-2-3: low, medium and severe staphylococcosis reported; ** NT: not typeable *S. aureus*;

*** only prevalent biotypes are displayed; **** breeders commercial source

To estimate whether the presence of a certain biotype has a consequence on production parameters the flock biotype percentage was compared with the productive parameters and with the lesion score using the linear regression. As shown in Table 2, a weak but significant association was found between mixed CV-C biotype flock percentage and mean number of weaned rabbits ($P=0.048$). No association was found, above chance, with other productive variables.

The high within mixed CV-C biotype flock prevalence (mean 43.9, 95% CI: 24.3% - 63.6%) could be explained by its ability to spread within the flock thanks to MSCRAMMs surface adhesins (Vancraeynest *et al.*, 2004).

Due to practice to discharge breeders with mastitis and abscesses it was not possible to find statistic proof for the correlation between mixed CV-C biotype percentage and the score given for lesions severity; despite that, all but two flock owners reported an anamnesis of staphylococcosis, giving support to the ability of this biotype to increase discharge of breeders due to mastitis and abscesses.

Flocks N. 6 and N. 11 with *S. aureus* mixed CV-C within flock prevalence of respectively 83.3% and 46.7% had no reported anamnesis of staphylococcosis. Preliminary data to be confirmed, indicate in those two strains genome the absence of genetic markers which are considered of importance in virulence ability of HV-SA strains (Vancraeynest *et al.*, 2007), it is possible those strains belong to a low virulence *S. aureus* group inside the mixed CV-C biotype as previously described (Devriese *et al.*, 1981).

Table 2: Regression coefficient from the linear regression analysis examining exposure to Mixed CV-C *S. aureus* biotype percentage

Variable	Estimated β coefficient	95% CI	P-value
Mean number birth rabbits	3.01	-8.62 14.64	0.581
Mean number of weaned rabbits	-22.30	-44.40 -0.20	0.048
Breeders replacement (%)	0.36	-0.32 1.04	0.270
Severity lesion score	0.24	-1.70 2.18	0.792

CONCLUSIONS

Our study pointed out a wide diffusion of *S. aureus* in the reproduction units of rabbit flocks in rational production systems and a mean inside flock prevalence of 77.6% was estimated in the selected sample. Three *S. aureus* biotypes were detected: the human one and two non host specific biotypes: mixed CV-C and mixed CV-A. Most flocks hosted just one biotype; in four flocks two biotypes were isolated: three flocks where one biotype was predominant and one unit where mixed CV-C and human biotypes were uniformly distributed. This quite dichotomic distribution of *S. aureus* biotypes seems to be correlated with the commercial source of breeders. Finally the statistic analysis of productive parameters relates the mixed CV-C *S. aureus* biotype to a low number of weaned rabbits. Results stress the necessity to apply an eradication policy to highly virulent *S. aureus* strains, moreover findings to be confirmed about the presence of low pathogen strains inside the mixed CV-C biotype point out the necessity to improve the classification of *S. aureus* with biomolecular methods and to validate methods for field screening of HV-SA.

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