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L. Meulemans, K. Hermans, L. Duchateau, F. Haesebrouck

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Authors: L. Meulemans, K. Hermans, L. Duchateau, F. Haesebrouck



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26 day 14 post-inoculation. The devised infection model is able to reliably reproduce the  
27 virulence properties of HV and LV *S. aureus* strains.

28

29 KEY WORDS: ***Staphylococcus aureus*; Rabbit; Skin; Infection model**

30

### 31 INTRODUCTION

32 Staphylococci are gram-positive, facultative anaerobic cocci, causing infections in humans  
33 and various animal species. *Staphylococcus aureus* is one of the most important pathogenic  
34 *Staphylococcus* species in veterinary medicine. In rabbits, it mainly causes skin infections,  
35 pododermatitis and mastitis with subsequent economic losses in industrial rabbitries. Within  
36 rabbit flocks two types of *S. aureus* infections occur. A first type, only affecting a small  
37 number of animals, is caused by low virulence (LV) strains. The second type of infection,  
38 caused by high virulence (HV) strains, has a major economic importance due to the epizootic  
39 spread of the infection throughout rabbitries and the chronic problems it induces (Hermans *et*  
40 *al.*, 2003).

41 Although resistance to antimicrobial agents in *S. aureus* from rabbits is relatively rare  
42 (Vancraeynest *et al.*, 2004a), antibiotic treatment of infected rabbit flocks is not effective.  
43 Vaccination against staphylococcosis is difficult to achieve (Hermans *et al.*, 2003; Lee, 1996).  
44 Previous studies (Hermans *et al.*, 2000b; 2001a; 2001b; Vancraeynest *et al.*, 2004b, 2006a)  
45 have already demonstrated specific differences in virulence factors and virulence-associated  
46 markers between high and low virulence strains, but the pathogenesis of those two types of *S.*  
47 *aureus* strains needs further investigation. Virulence genes such as the *bbp* and the selu-  
48 bearing *egc* locus have been indicated as putative virulence factors for HV *S. aureus* strains  
49 from rabbits (Vancraeynest *et al.*, 2004b, 2006a).

50 For studying bacterium–host interactions, determination of the role of the above mentioned  
51 virulence factors *in vivo*, and evaluation of vaccine efficacy, it is necessary to have a  
52 reproducible animal infection model. Up until now, a model reproducing the lesions caused  
53 by HV and LV *S. aureus* strains in rabbits, has not been described. It was the aim of this study  
54 to develop such a model.

55

## 56 MATERIAL AND METHODS

### 57 *Experimental animals*

58 The animal experiments were carried out following approval of the ethical committee from  
59 the Faculty of Veterinary Medicine of Ghent University. Forty-five nine-week-old albino  
60 hybrid rabbits (CLO, Melle, Belgium), weighing 2.0–2.5 kg and from either sex were used for  
61 experimental infections. The animals were kept individually in wire mesh cages and received  
62 food and water ad libitum. From each rabbit, the rectal temperature was measured before the  
63 onset of the experiment. The following body sites were sampled as described by Hermans et  
64 al. (1999): the nares, the auditory canal, the interdigital skin, the skin of the medial right  
65 foreleg, the axillar and inguinal skin region, the skin around the nipples, the perineum and the  
66 vagina or preputium. The samples were screened bacteriologically for the presence of HV *S.*  
67 *aureus* strains.

68

### 69 *S. aureus* strains

70 *S. aureus* strains used in this study were the isolates KH 24, KH 365, KH 171 (Hermans *et al.*,  
71 1999) and PI 41/95 (Devriese *et al.*, 1996) all obtained from Belgian rabbitries between 1995  
72 and 1999. The classification of the *S. aureus* strains into biotypes had been performed as  
73 described by Devriese (1984) and the phage typing was performed using bacteriophages of  
74 the international typing set for human *S. aureus* strains. Lytic reactions were examined at 100-

75 fold routine test dilution (Parker, 1962). Strain KH 24 belongs to the human biotype and to  
76 phage type 29/79/85/95 and strain KH 365 to the biotype–phage type combination mixed CV-  
77 C–52A/53/85. Both strains were isolated from healthy rabbits in rabbitries without chronic  
78 problems of staphylococcosis and are considered LV strains. Strain KH 171 was isolated from  
79 a rabbitry experiencing severe problems with mastitis, pododermatitis, pustular dermatitis and  
80 subcutaneous abscesses. This strain belongs to the biotype mixed CV-C and is sensitive to the  
81 phages of phage group II, namely 3A, 3C, 55 and 71. Strains of this type belong to a widely  
82 disseminated HV clone (Vancraeynest *et al.*, 2006b). Strain KH 171 demonstrated its  
83 colonisation capacities in previous intranasal experimental infections of rabbits (Hermans *et*  
84 *al.*, 2000a). Strain PI 41/95, showing biotype mixed CV-C and phage type  
85 29/79/42E/92/D11/HK2, is considered to be an atypical HV strain. It was isolated in 1995  
86 from a rabbitry with severe staphylococcosis (Devriese *et al.*, 1996). No strains of this type  
87 have been isolated since.

88 *S. aureus* strains were grown for 24 h at 37°C on Columbia agar (Gibco, Paisley, Scotland)  
89 containing 5% ovine blood in a 5% CO<sub>2</sub>-enriched environment. After checking the strains for  
90 purity, ten colonies of each strain were transferred into brain heart infusion (BHI) (Oxoid,  
91 Basingstoke, England) broth for 24 hours by 37°C whilst shaking.

92 The number of colony forming units (cfu) was determined by inoculation of 10-fold dilutions  
93 on blood agar. Bacterial suspensions were stored overnight at 4°C in BHI and prior to the  
94 experiment, the suspensions were centrifuged and the pellets adjusted to an appropriate  
95 concentration of 10<sup>9</sup> cfu / ml PBS.

96

#### 97 *Experimental Design*

98 The rabbits were randomly divided into four groups of 10 animals (A, B, C and D) and one  
99 group of 5 animals (N). Following inhalation anaesthesia with isoflurane (IsoFlo®, Abbott

100 Laboratories Ltd., Queensborough, England), the hair of both flanks was shaved with electric  
101 clippers (Arco, Moser, Germany) and disinfected thoroughly with 70% ethanol (Disinfectol®,  
102 Chem-Lab NV, Zedelgem, Belgium) during five minutes. After evaporation of the ethanol a  
103 tattoo pin (5 x 7mm) was pressed into the skin of both the left and the right flank with tattoo  
104 pincers (‘t Neerhof, Lokeren, Belgium). After each piercing the tattoo pin and the pincers  
105 were disinfected with ethanol. With these pincers, a 0.5 mm deep “O”-shaped dermal skin  
106 lesion was produced. The lesion on the right flank of the rabbits of groups A, B, C and D was  
107 inoculated with  $10^8$  cfu *S. aureus* bacteria in 0.1 ml PBS. *S. aureus* strains used were PI 41/95  
108 (group A), KH 171 (group B), KH 24 (group C) and KH 365 (group D), respectively. The  
109 lesions induced on the left flank were not inoculated in any of the rabbits. This was done to  
110 assess the capacity of the different strains to spread over the rabbit body to infect a sterile skin  
111 wound. In the rabbits of group N, lesions on the right flank were sham inoculated with 0.1 ml  
112 sterile PBS. After air-drying of the inoculum, the rabbits were allowed to recover from the  
113 anaesthesia.

114 Rabbits were inspected daily for the development of macroscopic skin lesions and the rectal  
115 temperature was measured. Skin lesions were measured by means of a vernier calliper. At 14  
116 days post-inoculation the animals were euthanased and swab samples of the nares and both  
117 flanks (abscesses) were collected for bacteriological analysis, as were skin biopsies for  
118 histopathological examination. From each inoculation group, random samples of the *S. aureus*  
119 strains isolated at the end of the experiment were taken for biotyping and phage typing.

120

### 121 *Bacteriological examination*

122 All samples were inoculated onto Columbia agar (Gibco, Paisley, Scotland) supplemented  
123 with 5% ovine blood. Samples of the nares and perineum were also cultured on modified  
124 Baird-Parker agar (Devriese, 1981). After 24 h incubation at 37°C, *S. aureus* colonies were

125 identified and classified into biotypes as described by Devriese (1984). Phage typing of the  
126 isolated *S. aureus* colonies was performed at the Pasteur Institute (Brussels, Belgium) using  
127 bacteriophages of the international typing set for human *S. aureus* strains according to Parker  
128 (1962).

129

### 130 *Histopathological examination*

131 Representative skin biopsies of the right and left flanks with underlying muscle were fixed in  
132 10% buffered formalin; paraffin embedded and cut into 5 µm slices. After conventional  
133 haematoxylin–eosin staining, tissue sections were evaluated using the light microscope.  
134 Histopathological lesions were classified according to the following parameters: crust  
135 formation, epidermal hyperplasia, degree of necrosis and type of inflammation in different  
136 dermal parts (superficial, mid and deep dermis) and the subcutis.

137

### 138 *Statistical analysis*

139 The different infection groups were compared with respect to the maximum observed abscess  
140 diameter and the area under the abscess diameter time curve (AUC). For animals euthanased  
141 before the end of the study period, the AUC was obtained through the LOCF (last observation  
142 carried forward) procedure assigning the value of the last observation for the remaining  
143 timepoints after euthanasia. A Kruskal Wallis test was used to compare results obtained with  
144 the four strains at a significance level of 0.05, followed by three pairwise comparisons based  
145 on the Wilcoxon rank sum test according to virulence type, namely low versus atypical, low  
146 versus high and atypical versus high, with a Bonferroni-corrected significance level of  
147 0.0133.

148 The abscess measurements were also compared between the different infection groups using a  
149 mixed model with animal as random effect and time and virulence and their interaction as

150 fixed categorical effects. Additionally, three pairwise comparisons were performed according  
151 to virulence type, namely low versus atypical, low versus high and atypical versus high, with  
152 a Bonferroni-corrected significance level of 0.0133.

153

## 154 **RESULTS**

### 155 *Clinical examination*

156 The rabbits remained alert and showed normal appetite and grooming activities throughout  
157 the whole experiment. Rectal temperatures stayed within the normal range (Harcourt-Brown,  
158 2002a) and varied between 38.5°C and 40.1°C.

159

### 160 *Macroscopic evaluation of lesions*

161 In none of the 45 rabbits was an abscess observed on the non-inoculated left flanks.  
162 From the rabbits of group A (inoculated with the atypical HV strain PI 41/95), nine out of ten  
163 animals developed an abscess on the right flank within 48 h post-infection. The diameter of  
164 the nodules varied between 10–20 mm and the lesions all reached their maximum diameter  
165 48–96 h after inoculation. Seven of these rabbits showed nodules larger than 15 mm in  
166 diameter and three of those showed lesions of 20 mm in diameter. At day 14 post-inoculation,  
167 nodules of 4–8 mm were observed in eight animals and one animal showed an abscess of 15  
168 mm at the end of the experiment. The skin of only one rabbit of group A showed a red  
169 discrete swelling 24 h post-infection with the formation of pustules after two days. After 72 h  
170 some dry crusts were noticed. During the experiment this rabbit did not develop other lesions.  
171 Except for one rabbit, all animals of group B (inoculated with the typical HV strain KH 171)  
172 developed abscesses on the right flank of at least 15 mm within 24–48 h after inoculation.  
173 Three rabbits had nodules of a minimum diameter of 35 mm after 24 h and these animals  
174 were euthanased three days post-inoculation when the diameters of the lesions had reached 40

175 mm. In the remaining animals, the maximum lesion diameter of 35 mm was attained between  
176 day 5 and day 7 of the study with nodule diameters ranging from 20–40 mm. One rabbit  
177 developed an abscess of 20 mm after 24 h that started to regress after six days to enlarge again  
178 at day 13 finally reaching its maximum diameter of 30 mm on the last day of the experiment.  
179 One rabbit only showed a pustular dermatitis with a diffuse red skin rash throughout the  
180 whole study. On day 14 an abscess of 12 mm was noted. In one animal from group B, a small  
181 abscess of 2 mm appeared on the lower lip on day six. This abscess retracted and at the end of  
182 the study a similar nodule was noted on the upper lip adjacent to the first nodule.  
183 From group C (inoculated with the LV strain KH 24), seven rabbits out of ten developed skin  
184 nodules on the right flank of a maximum diameter of 7 mm in the first 72 h of the study. At  
185 the end of the experiment, the skin of six of the rabbits was healed (alopecic zone with dry  
186 crusts or scales). The remaining four rabbits showed a skin nodule of 3–7 mm in diameter.  
187 Eight rabbits from group D (inoculated with the LV strain KH 365) showed abscess formation  
188 on the right flank in the first three days post-infection with the diameter of the abscesses  
189 reaching not more than 7 mm. Two rabbits showed lesions of 12 and 20 mm, respectively. At  
190 the end stage of the study no more skin lesions were observed in six rabbits. Four rabbits  
191 presented abscesses of 4–10 mm in diameter.  
192 The skin of the sham-inoculated control group remained “healthy” during the experiment.  
193 A graphic overview of the mean abscess diameters in the different infection groups is  
194 provided in Figure 1.

195

#### 196 *Bacteriological examination*

197 HV *S. aureus* was not isolated from the samples taken before the onset of the experiment.  
198 After euthanasia, of the ten rabbits infected with the atypical HV strain PI 41/95 (group A),  
199 one was found positive for *S. aureus* in the nose, four were positive on the inoculated right

200 flank and one on the non-inoculated left flank. Of the rabbits of group B, infected with the  
201 typical HV strain KH 171, three were positive for *S. aureus* in the nose, all ten rabbits were  
202 positive on the right flank and seven were positive on the left flank. In the rabbits of group C,  
203 experimentally infected with the LV strain KH 24, *S. aureus* was not isolated from the nose or  
204 from the left flank, whilst two of the ten animals were positive for *S. aureus* on the right  
205 flank. In rabbits inoculated with the LV strain KH 365 (group D), one animal showed  
206 presence of *S. aureus* in the nose, one on the left flank, and two on the right flank.  
207 In all groups, no changes were noted in the biotype or in the phage type of the isolates  
208 obtained at the end of the experiment compared to the strains used for inoculation.

209

#### 210 *Histopathological examination*

211 All 45 skin biopsies from the non-inoculated left flank of the rabbits and the biopsies of the  
212 right flank of the sham inoculated animals (group N) showed a similar histology aspect and  
213 were considered as normal according to the description by Miltzer (1982). The epidermis was  
214 composed of 4–6 cellayers and the dermis contained loose collagen fibres (fibroblasts) and  
215 few polymorphonuclear leucocytes (PMN's) in the superficial part.

216 In group A (inoculated with the atypical HV strain PI 41/95), three different inflammation  
217 patterns could be distinguished. Four rabbits showed a mild inflammation of the dermis; one  
218 of them showed a mild focal superficial perivasculitis with infiltration of PMN's, but no  
219 changes were noted in the epidermis or deeper dermal parts. The three remaining samples  
220 showed a mild epidermal hyperplasia with a mild multifocal polymorphonuclear perivascular  
221 dermatitis (superficial, mid and deep dermis) with evidence of nuclear debris and a diffuse  
222 mild fibrosis. Four rabbits showed a moderate epidermal hyperplasia, moderate fibrosis of the  
223 dermis with follicular atrophy, a moderate multifocal to diffuse perivascular dermatitis with  
224 infiltration of PMN's and macrophages and a mild inflammation of the subcutis. In one case

225 serocellular crust formation was noted with evidence of necrotic PMN's and keratin. Two  
226 rabbits of group A showed a well demarcated and organised abscess in the dermis with  
227 peripheral fibrosis, epidermal hyperplasia with focal blurring of the basal membrane and mild  
228 interface dermatitis with infiltration of PMN's and lymphocytes, a diffuse cell-rich superficial  
229 and deep perivascular dermatitis with mild oedema. In one of these two rabbits there was  
230 crust formation and evidence of perifolliculitis and intraluminal suppurative folliculitis.  
231 Two out of ten rabbits in group B (inoculated with the typical HV strain KH 171) showed  
232 marked dermal fibrosis, epidermal hyperplasia and a mild superficial and deep suppurative  
233 vascular dermatitis. In the fibrotic areas there was a lack of follicular and adnexal structures.  
234 Eight rabbits showed an extensive area of dermal necrosis with focal loss of the epidermis and  
235 crust formation. The necrotic area was demarcated by lytic collagen fibres, a massive  
236 infiltration of PMN's and marked oedema. The biopsies showed evidence of perifolliculitis,  
237 infiltrative mural folliculitis, furunculosis and myositis. Three of these eight rabbits (those  
238 euthanized at day three post-inoculation) showed similar but more fulminant lesions with  
239 severe lysis of dermal and epidermal components, subepidermal haemorrhages, massive  
240 infiltration of degranulating PMN's and multifocal clusters of bacteria, suppurative myositis  
241 and deep dermal oedema. The slides of the lip nodules that appeared in one animal showed a  
242 dermal fibrosis in the lower lip and a nodular suppurative dermatitis in the upper lip.  
243 In group C (inoculated with the LV strain KH 24), three out of ten animals showed mild  
244 superficial focal perivascular dermatitis with mild fibrosis. Four rabbits showed epidermal  
245 hyperplasia with mild intracellular oedema of the stratum spinosum and stratum granulosum,  
246 a mild diffuse superficial perivasculitis, moderate to marked dermal fibrosis with focal  
247 perivascular dermatitis and follicular atrophy. Three out of ten rabbits showed mild  
248 superficial perivascular dermatitis with infiltration of PMN's and monocytes and a  
249 demarcated area of central necrotic cellular debris and PMN's surrounded by dense collagen

250 fibres and infiltrating macrophages. There was deep dermal fibrosis with mild focal  
251 perivascular dermatitis.

252 From group D (inoculated with the LV strain KH 365), two skin biopsies showed mild focal  
253 epidermal hyperplasia and a mild superficial cellular infiltration consisting mostly of  
254 monocytes and lymphocytes and some PMN's. Two slides showed moderate epidermal  
255 hyperplasia, multifocal moderate dermal infiltrates of monocytes and lymphocytes, some  
256 PMN's and focal single cell necrosis. There was extravasation of red blood cells. Six out of  
257 ten rabbits showed dermal fibrosis and well-demarcated organised abscesses surrounded by  
258 multinuclear giant cells and macrophages phagocytosing bacteria, PMN's and  
259 erythrophagocytosis.

260

261 *Statistical analysis*

262 For the maximum abscess diameter, results obtained for the four strains differed significantly  
263 ( $P < 0.0001$ ) with the median (range) equal to 15 mm (2–20) for group A, 32.5 mm (12–45) for  
264 group B, 7 mm (0–8) for group C and 7 mm (0–20) for group D. LV strains produced a  
265 significantly lower maximum abscess diameter than did the atypical strain ( $P = 0.0006$ ) and  
266 than the HV strain ( $P < 0.0001$ ), and the atypical strain produced a significantly lower  
267 maximum abscess diameter than the HV strain ( $P = 0.0025$ ).

268 For the AUC, the four strains differed significantly ( $P < 0.0001$ ) with the median (range) equal  
269 to 112.5 mm<sup>2</sup> (4–234) for group A, 282 mm<sup>2</sup> (46–561) for group B, 43 mm<sup>2</sup> (0–101) for group  
270 C and 75 mm<sup>2</sup> (0–176) for group D. LV strains had a significantly lower AUC than the  
271 atypical strain ( $P = 0.0037$ ) and than the HV strain ( $P = 0.0002$ ), and the atypical strain had a  
272 significantly lower AUC than the HV strain ( $P = 0.0041$ ).

273 In the mixed model, both time, strain and their interaction differed significantly. For pairwise  
274 comparisons, however, the LV versus the atypical strain did not differ significantly ( $P = 0.185$ ).

275 The LV versus the HV strain ( $P < 0.0001$ ) and the atypical versus the HV strain ( $P < 0.0001$ ) did  
276 differ significantly from each other.

277

## 278 **DISCUSSION**

279 Many attempts have been made to induce experimental *S. aureus* skin infections with and  
280 without traumatising the skin (Marples and Kligman, 1975; Akiyama *et al.*, 1996; Gisby and  
281 Bryant, 2000). It is generally accepted that staphylococci colonise the normal skin and  
282 mucosae of humans and animals and invade the deeper dermal tissues by penetrating through  
283 a skin defect. *S. aureus* infection models that use intact skin (Marples and Kligman, 1975) are  
284 difficult to reproduce and require large inocula and complete skin occlusion. However, when  
285 the epidermal barrier is breached, the reproducibility of the skin infection model and its  
286 resemblance to naturally occurring infections increases. This breach of the epidermis can be  
287 obtained by minimal trauma, for example with cut wounds or abrasions of the skin (Akiyama  
288 *et al.*, 1996; Gisby and Bryant, 2000). Other models bypass the epidermal barrier by injecting  
289 the bacterial inoculum directly intradermally or subcutaneously (Reinosa *et al.*, 2002). Some  
290 studies mention the use of a surgical skin incision followed by injection of the bacteria in the  
291 submuscular space (Palma *et al.*, 1996) or subcutaneously (Chavakis *et al.*, 2002) or by  
292 inserting inoculated sutures in the inflicted wound (Nicholas *et al.*, 1999; Gisby and Bryant,  
293 2000). In murine skin air sac models, slow subdermal injection of air and bacteria minimize  
294 the risk of environmental contamination (Raeder and Boyle, 1993). Most previously described  
295 methods are quite different from the natural way of infection, however. Rabbit skin is thin and  
296 easily traumatised (Harcourt-Brown, 2002b). In rabbits, skin excoriations may occur due to  
297 management conditions, such as a rough grid floor of rabbit cages, fighting between animals  
298 and possibly during auto- and allogrooming activities. The infection model used in the present

299 study aimed at mimicking the natural infection route of *S. aureus* in rabbits, namely through  
300 these skin wounds.

301 When comparing the results of the macroscopic examination of different groups it is clear that  
302 rabbits inoculated with the HV *S. aureus* strain KH 171 developed larger abscesses with a  
303 more acute onset and a slower involution compared to those inoculated with LV strains. The  
304 LV strains KH 24 and KH 365 induced lesions, but in only one out of twenty rabbits was an  
305 abscess seen (maximum size of 20 mm). In nine out of ten rabbits inoculated with the typical  
306 HV strain, abscesses with diameters between 20–40 mm developed. This demonstrates that  
307 the present skin infection model is able to reproduce the virulence differences between HV  
308 and LV rabbit *S. aureus* strains seen under natural conditions.

309 At the endstage of the experiment, biopsies taken from the groups infected with LV strains  
310 clearly showed that there was only a very mild residual cellular reaction in the dermis and in  
311 some cases there was healing of the skin through scarring or fibrosis of the dermal tissue. The  
312 skin of a normal healthy rabbit is apparently capable of curing itself from a high dose of LV  
313 *S. aureus* strains. Indeed, at the time of euthanasia, only two out of ten rabbits in both groups  
314 inoculated with LV strains still showed the presence of *S. aureus* on the site of inoculation,  
315 while in contrast the typical HV strain was isolated from the inoculation site of all ten rabbits  
316 infected with this strain.

317 Strain PI 41/95 has been described to induce severe staphylococcosis lesions in rabbits such  
318 as rapidly evolving abscesses with a predilection for muzzle, toes and perianal region  
319 (Devriese *et al.*, 1996). This strain, however, did not induce the expected lesions in our skin  
320 infection model. Moreover, since 1995, strains of this type have not been isolated anymore. It  
321 may therefore be possible that the strain lacks some virulence factors compared to the typical  
322 HV strains. Virulence genes that have already been documented in the typical HV but not in  
323 the atypical HV strain are the *bbp* gene, encoding bone sialoprotein binding protein, and the

324 *egc* cluster, encoding different enterotoxins and enterotoxin-like proteins with superantigen  
325 properties (Vancraeynest *et al.*, 2004b; 2006a). However, their exact role in the pathogenesis  
326 of infections with typical HV strains remains to be elucidated. The skin infection model  
327 developed in the present study may contribute to this research.

328 Previous experimental intranasal inoculation studies (Hermans *et al.*, 1999) showed that  
329 typical high virulence strains have an increased capacity to colonise host epithelia and the  
330 ability to spread to different body sites. This was confirmed in the present studies. Indeed the  
331 typical HV strain was isolated from the non-inoculated left flank and the nares at the end of  
332 the experiment in seven and three out of ten rabbits, respectively. In contrast, the atypical HV  
333 strain and the LV strains were isolated from these body sites in only one animal.

334 Although the HV *S. aureus* strain was isolated from the skin of the left flank in seven out of  
335 ten rabbits of group B at the time of euthanasia, the experimentally induced skin trauma on  
336 the left flank did not become infected in any rabbit and no macroscopical and histological  
337 changes were noted. It is possible that the induced lesion, which was not deeper than 0.5 mm,  
338 had healed before the *S. aureus* inoculated on the other flank spread to this body site.

339 It was noted that in one animal from group B, inoculated with the typical HV strain KH 171, a  
340 small abscess of 2 mm appeared on the lower lip. This abscess retracted and at the end of the  
341 study, the animal showed a new similar nodule on the upper lip adjacent to the first nodule.

342 This indicates that HV *S. aureus* strains may have the capacity to induce lesions on apparently  
343 intact skin. The same finding was noticed in a preliminary infection experiment (unpublished)  
344 with the same HV strain. The lip infections are possibly due to grooming and licking activities  
345 of the rabbit.

346 The histological lesions seen in the present study are similar to those described in the  
347 literature for staphylococcal infections of other animal species. *Staphylococcus* spp. are  
348 frequently isolated from superficial and deep folliculitis, furunculosis and cellulitis in dogs

349 and subcutaneous abscesses in cats. Histological features of such superficial infections are  
350 mainly associated with a suppurative interstitial dermatitis, perifolliculitis and perifollicular  
351 fibrosis. Deep infections show an initial infiltration of neutrophils that can become  
352 granulomatous in chronic cases (Scott *et al.*, 2001; Gross *et al.*, 2005). Coagulase-positive  
353 staphylococci are often the cause of bacterial pseudomycetoma, an under-diagnosed disease in  
354 many animal species (Scott *et al.*, 2001). Histopathological features are a nodular to diffuse  
355 dermatitis and/or panniculitis with compact bacteria colonies surrounded by an infiltrate of  
356 histiocytes, plasma cells, lymphocytes, neutrophils and multinucleate histiocytic giant cells  
357 similar to the lesions found in the rabbits of group C and D herein.

358 In conclusion, the present model was able to reproduce *S. aureus* skin infections in rabbits  
359 similar to those seen in the field. Furthermore, there was a significant difference between HV  
360 and LV strains in the size and gravity of the developed abscesses, thereby confirming that the  
361 virulence differences noticed in the field can also be reproduced under laboratory conditions.  
362 This infection model is thus suitable for further rabbit *S. aureus* research. It has also a  
363 potential for other *S. aureus* research, for instance in the field of MRSA or *S. aureus* isogenic  
364 mutant strains.

365

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370

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478 and D, ●), throughout the experiment (with vertical bars representing the standard deviation).

