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1 **QUORUM SENSING IN VETERINARY PATHOGENS: MECHANISMS, CLINICAL**
2 **IMPORTANCE AND FUTURE PERSPECTIVES**

3
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5
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9
10 **ABSTRACT**

11
12 Under certain circumstances the individuals of a bacterial population may find advantages in
13 acting together and making “collective decisions”. This phenomenon is better known as
14 quorum sensing. When the concentration of signal molecules produced by the surrounding
15 bacteria exceeds a certain threshold, the bacterial population acts as a single organism,
16 collectively expressing virulence genes, biofilm forming genes, etc. Several mechanisms of
17 quorum sensing are discussed, each with its distinct signal molecules and respective receptors.
18 Some of these mechanisms are restricted to sensing intraspecies signalling, but interspecies
19 and even interkingdom signalling have also been described. Several veterinary pathogens
20 such as *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa*
21 and *Salmonella* Typhimurium use quorum sensing as a means to optimize virulence gene
22 expression and host colonization. Therefore, targeting of the QS mechanisms may provide a
23 novel strategy for combating bacterial infections, also in veterinary medicine.

24
25 Key words: quorum sensing, signal molecules, veterinary pathogens

26

27 INTRODUCTION

28

29 The communication mechanism that enables bacteria to make ‘collective decisions’ is
30 called ‘quorum sensing’. For this purpose, small chemical signal molecules are synthesized
31 and secreted into the environment. By using specific detection systems to sense the
32 concentration of these signal molecules, the surrounding bacteria are aware of the bacterial
33 cell density. When the signal molecule concentration reaches a critical threshold, the gene
34 expression and, as a result, the behaviour of the total population is changed (Figure 1).

35 Quorum sensing (QS) was first described in the 1970s, when Nealson and colleagues
36 (1970) investigated the bioluminescence of *Vibrio fischeri* and *Vibrio harveyi*. Ever since, QS
37 has been assigned a role in various mechanisms of gene regulation: biofilm formation
38 (Haesebrouck et al., 2007; Irie and Parsek, 2008), virulence adaptation (Salmond et al., 1995;
39 Vendeville et al., 2005), the production of antimicrobial substances (Salmond et al., 1995;
40 Kleerebezem, 2004), motility (Bearson and Bearson, 2007; Atkinson et al., 2008), sporulation
41 (Lazazzera, 2000), etc. Although these mechanisms have mostly been studied in non-
42 pathogenic bacteria or in human pathogens, the last few years, more studies have been
43 conducted focusing on bacterial species of veterinary importance.

44 In this manuscript, the different mechanisms of QS, some examples of the exploitation
45 of QS by bacterial pathogens of veterinary importance, and possible applications in future
46 veterinary medicine are reviewed.

47

48

49 MECHANISMS OF QUORUM SENSING

50

51 **Quorum sensing in Gram positive bacteria: autoinducing polypeptides**

52

53 Gram positive bacteria mainly use post-translational modified oligopeptides as QS
54 signal molecules (Lyon and Novick, 2004). These autoinducing polypeptides (AIPs) are made
55 up of 5 to 17 amino acids with side chain modifications consisting, for example, of isoprenyl
56 groups (*Bacillus* spp.) or thiolactone rings (*Staphylococcus* spp.) (Ji et al., 1997; Lyon and
57 Novick, 2004). The AIPs are synthesized in the cytoplasm as precursor peptides, and then
58 cleaved, modified and transported to the extracellular environment (Lyon and Novick, 2004).
59 In the surroundings of the producing bacterial cell, the AIPs can be detected by membrane-
60 bound two-component detection systems. Detection of the AIPs by the sensorial part of the
61 two-component systems leads to phosphorylation and therefore activation of the response
62 regulator protein (Lyon and Novick, 2004; Waters and Bassler, 2005). This response protein
63 interacts with the target promoter DNA and the expression of the QS regulated genes is
64 switched on (Figure 2).

65 The structure of the signal peptide varies between different Gram positive species.
66 Since the binding efficiency on the receptors is highly dependent on the chemical structure of
67 the AIPs, it is generally accepted that mainly Gram positive, but also some Gram negative
68 bacteria use this system for intraspecies communication (Goerke et al., 2003; Dirix et al.,
69 2004; Novick and Geisinger, 2008).

70

71 **Quorum sensing in Gram negative bacteria: N-acylhomoserine lactones**

72

73 In Gram negative bacteria, the signal molecules are N-acylhomoserine lactones
74 (AHLs), which are also called autoinducer 1 (AI-1). They consist of a conserved homoserine
75 lactone ring which is connected to an acyl side chain of variable length (4 to 18 carbon atoms)
76 and variable extra modification. AHLs with a short acyl side chain can diffuse passively in
77 and out the bacterial cell (Moré et al., 1996; Fuqua et al., 2001). This is in contrast with AHLs
78 with a long acyl side chain and the AIPs, which need active transport mechanisms to cross the
79 bacterial cell membrane (Fuqua et al., 2001; Novick and Geisinger, 2008).

80 LuxI homologous proteins play a major role in the synthesis of the AHLs, which
81 increase in concentration as the cell density increases. At a critical concentration, LuxR
82 homologues bind the signal molecules and subsequently regulate gene transcription (Fuqua et
83 al., 2001; Waters and Bassler, 2005) (Figure 2). It is becoming more and more obvious,
84 however, that there is a considerable diversity in the way in which the LuxI and LuxR
85 homologues from different bacterial species operate (Smith et al., 2006). Since the LuxR type
86 proteins only detect specific AHLs, this QS system is also mainly used for intraspecies
87 communication. Nevertheless, LuxR type proteins that are able to detect several AHLs have
88 been described. For example, SdiA of *Salmonella* is believed to be mainly involved in
89 interspecies bacterial communication (Walters and Sperandio, 2006).

90

91 **Quorum sensing in both Gram positive and Gram negative bacteria: autoinducer 2**

92 In the late 1990s, a second QS system was discovered in various Gram negative
93 bacteria (Surette et al., 1999). In this system, the autoinducer (AI-2) is a furanosyl borate ester
94 that is formed by the LuxS protein (Waters and Bassler, 2005). To date, AI-2 has been
95 described in more than 50 different bacterial species, comprising both Gram positive and
96 Gram negative bacteria (Surette et al., 1999; Schauder et al., 2001; Hardie and Heurlier,
97 2008). In *Vibrio harveyi*, the receptor for the AI-2 molecules is the periplasmatic LuxP

98 protein. The LuxP-AI-2 complex binds to a second protein, LuxQ, which is a two-component
99 protein that contains both a sensor kinase domain and a response regulator domain (Neiditch
100 et al., 2005). In low bacterial concentrations, and therefore in the absence of the AI-2, LuxQ
101 phosphorylates LuxO with the aid of the LuxU intermediary protein, which in turn activates
102 the transcription of a repressor protein blocking the transcription of the luciferase operon. At
103 high bacterial densities, the AI-2 presence induces the phosphatase activity of LuxQ, which
104 inactivates LuxO, resulting in the LuxR mediated transcription of the luciferase operon
105 (Neiditch et al., 2005) (Figure 2). The LuxR component of this system should not be confused
106 with the LuxR protein of *Vibrio fischeri* (see above). The autoinducer AI-2 could serve as a
107 ‘universal signal’ for interspecies communication (Xavier and Bassler, 2003), though in most
108 enteric bacteria the genes that are directly regulated by AI-2 code for an ABC transporter
109 system and it has been suggested that the role of AI-2 is mainly metabolic (Vendeville et al.,
110 2005; Walters and Sperandio, 2006; Rezzonico and Duffy, 2008).

111

112 **Quorum sensing beyond bacterial borders: autoinducer 3**

113 Enterohaemorrhagic *Eschericia coli* (EHEC) is the cause of various food-borne
114 outbreaks of severe intestinal disease and the causative agent of the hemolytic uremic
115 syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in humans (Moxley et al.,
116 2004; Karch et al., 2005). EHEC motility and attaching and effacing virulence genes are
117 regulated by a QS mechanism (Moxley et al., 2004) that involves the poorly characterized
118 auto inducer 3 (AI-3), which is produced by several enteric bacterial species (Kendall and
119 Sperandio, 2007). Since epinephrine/norepinephrine induce the same virulence gene
120 expression in EHEC and since the effects of AI-3 can be inhibited by adrenergic receptor
121 antagonists, AI-3 probably is structurally similar to epinephrine/norepinephrine (Sperandio et
122 al., 2003). In addition, the presence of epinephrine/norepinephrine itself may serve as a QS

123 signal. Apart from the EHEC QseE/F two-component system, QseB/C has been identified as
124 the receptor/response regulator of both AI-3 and epinephrine/norepinephrine in a number of
125 bacterial species (Clarke et al., 2006; Walters and Sperandio, 2006) (Figure 2). Therefore, this
126 adrenergic receptor is thought to play a role in interkingdom signaling (Kendall and
127 Sperandio, 2007).

128

129 CLINICAL SIGNIFICANCE OF QUORUM SENSING IN VETERINARY PATHOGENS

130

131 *Staphylococcus aureus*

132 The *agr* (accessory gene regulator) QS system in *Staphylococcus aureus* (*S. aureus*) is
133 one of the most studied QS mechanisms in Gram positive bacteria. Using sequence analysis,
134 *S. aureus* can be divided into 4 different *agr* specific groups. The AIPs of one *agr* group
135 inhibit the expression of the *agr* expression of the other groups (Novick, 2003). Several
136 haemolysins, enterotoxins, exfoliating toxins, enzymes and surface proteins are regulated by
137 *agr*. Therefore *agr* has been assigned various functions in the pathogenesis of *S. aureus*
138 infections in several animal species.

139 In rabbits, *S. aureus* infections lead to skin infections, mastitis and internal
140 abscessation. Low virulent strains may occasionally infect an individual animal, while highly
141 virulent strains are the cause of chronic problems and they spread rapidly throughout the
142 rabbitry (Vancraeynest et al., 2006). It has been shown that highly virulent strains are clonal
143 and grouped into *agr* type IV (Vancraeynest et al., 2006). In addition, it has been shown that
144 *agr* deletion mutants are attenuated in their capacity to induce endocarditis, osteomyelitis and
145 endophthalmitis in rabbits (Cheung et al., 1994; Gillaspay et al., 1995; Booth et al., 1995).

146 *S. aureus* is worldwide the most frequently isolated bacterium from bovine
147 intramammary infections. In contrast to highly virulent rabbit associated *S. aureus* isolates,

148 strains from bovine mastitis cases are predominantly classified in *agr* group I and to a lesser
149 extent in *agr* groups II and III (Buzzola et al., 2007; Vautor et al., 2008). Bovine *S. aureus*
150 strains belonging to *agr* group I show significantly increased capacities to be internalized in
151 bovine mammary epithelial cells and persist in higher numbers in murine mammary glands
152 (Buzzola et al., 2007), while *agr* group II strains might prefer the extracellular niche and
153 therefore might be more dependent on biofilm formation (Melchior et al., 2009). In addition,
154 it has been suggested that penicillin resistance in bovine *S. aureus* strains is correlated to the
155 presence of *agr* group I genes (Melchior et al., 2009).

156 In contrast, for example, to *Pseudomonas aeruginosa*, biofilm formation in *S. aureus* is
157 negatively regulated by the main QS system. *Agr* dependent expression contributes to the
158 detachment of biofilms and subsequent colonization of new sites (Boles and Horswill, 2008).
159 Therefore, although still speculative, the QS dependent detachment from biofilms may render
160 some *S. aureus* strains more capable of spreading through a herd and causing chronic
161 problems at the herd level (e.g. highly virulent rabbit strains). This may also explain why
162 *agr*⁺ and *agr*⁻ variants of *S. aureus* might have a cooperative interaction in certain types of
163 infections (Traber et al., 2008).

164

165 ***Staphylococcus pseudintermedius***

166 Staphylococcal strains formerly designated as *Staphylococcus intermedius* (*S.*
167 *intermedius*) are currently assembled in the so-called *S. intermedius* group (SIG), consisting
168 of *S. intermedius*, *Staphylococcus pseudintermedius* (*S. pseudintermedius*) and
169 *Staphylococcus delphini* (*S. delphini*) (Hajek, 1976; Varaldo et al., 1988; Devriese et al.,
170 2005; Devriese et al., 2009). From a clinical point of view, *S. pseudintermedius* is probably
171 the most significant species of the SIG, being an important canine opportunistic pathogen,
172 often isolated from dermatitis, otitis and other secondary infections in dogs (Bannoehr et al.,

173 2007). Since *S. pseudintermedius* produces toxins analogous to the *agr* dependend toxins of *S.*
174 *aureus*, it has been hypothesized that the *S. pseudintermedius* toxins are regulated by a
175 comparable QS mechanism (Sung et al., 2006). However, the *Staphylococcus* spp. of the SIG
176 differ from all other *Staphylococcus* spp., including *S. aureus*, in the fact that their AIP
177 contains a lactone in stead of a thiolactone ring (Dufour et al., 2002; Ji et al., 2005; Novick
178 and Geisinger, 2008). This nonapeptide shows both auto-inducing and cross-inhibiting
179 activities (Ji et al., 2005). DNA sequencing revealed the presence of four predicted AIP
180 variants within the SIG (Bannoehr et al., 2007). Novick and Geisinger (2008) suggest that the
181 divergence of the lactone containing AIP of the SIG from the thiolactone containing AIP of *S.*
182 *aureus*, may be correlated to the divergence of *S. pseudintermedius* towards the canine host.
183 However, the fact that no association could be made between the AIP variants of the SIG and
184 the different host species, disease status of the host, clinical or geographic origin of the strains
185 might nuance this hypothesis (Bannoehr et al., 2007).

186

187 ***Pseudomonas aeruginosa***

188 *Pseudomonas aeruginosa* (*P. aeruginosa*) is an important opportunistic pathogen in
189 human and veterinary medicine. *Pseudomonas* infections are difficult to treat since this
190 bacterium is resistant to several antimicrobial agents (Rubin et al., 2008; Weese, 2008) and its
191 antimicrobial susceptibility is even lower when it is present in biofilms (Haesebrouck et al.,
192 2007; Girard and Bloemberg, 2008). *P. aeruginosa* may cause various tissue infections in
193 different animal species, which illustrates the adaptability of this bacterium. A multitude of
194 virulence factors are regulated by at least one of the 2 different QS systems present in *P.*
195 *aeruginosa*: Las and Rhl (Whiteley et al., 1999; Girard and Bloemberg, 2008).

196 *Pseudomonas* is notorious for its capability to form biofilms. Through the formation of
197 a biofilm, the bacteria are able to colonize all kinds of animal tissues, plants and inert

198 surfaces, thereby increasing environmental persistence (Haesebrouck et al., 2007). In addition,
199 bacteria inside a biofilm are difficult to kill either by antimicrobial substances or by host
200 defense mechanisms, and therefore often result in chronic infections that do not respond to
201 antimicrobial therapy. Since *P. aeruginosa* is found ubiquitously in water, wet surfaces are
202 predisposed to be colonized with a *P. aeruginosa* biofilm. In veterinary medicine, the biofilm
203 producing capability of *P. aeruginosa* is most feared in cases of infections involving invasive
204 devices or implants (Weese et al., 2008). *P. aeruginosa* is also a severe complicating factor in
205 cases of equine ulcerative keratitis (Keller and Hendrix, 2005), in dermatitis, otitis externa
206 and urinary tract infections in small animals (Rubin et al., 2008), in fleece rot in sheep
207 (Kingsford and Raadsma, 1997), in bovine mastitis, which is often associated with
208 contaminated udder washing water or contaminated intramammary dry-cow preparations
209 (McLennan et al., 1997), and in hemorrhagic pneumonia in mink (Hammer et al., 2003).

210 Even though QS often favors the expression of virulence genes, Tron et al. (2004)
211 showed that *P. aeruginosa* canine otitis externa isolates share a deficiency of one or more
212 components of the Rhl QS system, leading to the loss of the elastase virulence factor. The
213 virulence factor that favors colonization of canine ears may be under the negative control of
214 the QS system, or a factor that facilitates removal of the bacterium from the canine ears may
215 be under the positive control of Rhl. The authors postulate that either this phenotype might be
216 present before infection or else it might be acquired after infection in an effort to adapt to the
217 novel environment. Recent research suggests an alternative explanation for the presence of
218 QS deficient strains on sites where it can be expected that biofilm formation should be
219 favourable for colonization. Evolutionary biologists describe the phenomenon of ‘cheating’
220 individuals in the population (Dunny et al., 2008). Such cheaters profit from the benefits of
221 the cooperating population, without contributing to the community. Since these cheaters do
222 not invest in the production of the biofilm, they have a competitive advantage relative to the

223 biofilm producing individuals and, as a consequence, can become predominant in the
224 community. However, when the proportion of cheaters becomes too large, this can affect the
225 stability of the biofilm and the entire population may crash as a result (Dunny et al., 2008).

226

227 *Salmonella*

228 Several QS systems have been described in *Salmonella*. *Salmonella* has a LuxR
229 homologue, SdiA, but does not have a luxI homologue and therefore does not produce AHLs
230 (Walters and Sperandio, 2006). It has been suggested that SdiA senses AHLs produced by
231 other bacterial species (Michael et al., 2001) and in this way senses the presence of the
232 intestinal environment. SdiA regulates the expression of virulence genes with different
233 functions, ranging from resistance to complement killing to the expression of fimbriae. In
234 murine, poultry or bovine models, however, an *sdiA* mutant strain is not attenuated and, very
235 recently, SdiA dependent gene expression was shown to be activated in the gut of turtles, but
236 not in the intestines of several homoiothermic animal species (Smith et al., 2008). The role of
237 SdiA in *Salmonella* pathogenesis therefore still remains largely unknown.

238 In *Salmonella enterica* subspecies *enterica* serotype Typhimurium (*Salmonella*
239 Typhimurium), the *luxS* gene is directly involved in the AI-2 synthesis. It was recently
240 demonstrated that *luxS* and the *Salmonella* AI-2, 4,5dihydroxy-2,3-pentanedione (DPD), are
241 necessary for *Salmonella* virulence (Choi et al., 2007). Deletion mutants lacking the *luxS* gene
242 are severely attenuated in the expression of the virulence genes of the *Salmonella*
243 Pathogenicity Island 1 (SPI-1). These genes are required for the efficient invasion of intestinal
244 epithelial cells and are therefore crucial in the pathogenesis of *Salmonella* infections in a wide
245 variety of animal hosts, including poultry, pigs and cattle (Morgan et al., 2004; Bohez et al.,
246 2006; Boyen et al., 2006). This finding suggests that *Salmonella* might secure the initial

247 infection process by producing SPI-1 virulence factors only when its population size attains a
248 high enough level (Choi et al., 2007).

249 Eukaryotic cells communicate with each other using various hormones or hormone-
250 like substances, for example epinephrine and norepinephrine. These signals can be misused,
251 however, by bacterial pathogens, such as *Salmonella*, to colonize the host or to induce disease
252 (Walters and Sperandio, 2006). Catecholamines may stimulate *Salmonella* Typhimurium by
253 two mechanisms: as a quorum sensing signal or as a supplier of iron (Bearson et al., 2008). In
254 *Salmonella*, the QseC AI-3 sensor, which is able to detect norepinephrine, has also been
255 implicated in the regulation of virulence traits such as motility and *in vivo* competitive fitness
256 in pigs (Bearson and Bearson, 2008). Even though the role of catecholamines or even other
257 neurotransmitters or hormones in the pathogenesis of *Salmonella* infections is still in its
258 infancy, the ability to respond to norepinephrine produced by the host has potential food
259 safety consequences. The mechanism described above may be one of the factors causing the
260 sudden burst of *Salmonella* excretion by stressed pigs, for example, during transport to the
261 slaughter house (Bearson and Bearson, 2008).

262

263 **Other veterinary pathogens**

264

265 It has been shown that several Pasteurellaceae, including *Mannheimia haemolytica*,
266 *Pasteurella multocida*, *Bibersteinia* (formerly *Pasteurella*) *trehalosi*, *Actinobacillus suis* and
267 *Actinobacillus pleuropneumoniae*, contain LuxS homologues in their genome and are able to
268 produce AI-2-like molecules (Malott and Lo, 2002). Also in *Histophilus somni*, QS may
269 contribute to pathogenesis (Corbeil, 2007). A luxS deletion mutant in *Mannheimia*
270 *haemolytica* did not show altered leukotoxin expression levels, but had increased expression
271 levels of putative virulence genes such as a transferrin binding protein, an adhesin and capsule

272 biosynthesis, and was outcompeted by the wild type parent strain in a calf challenge model
273 (van der Vinne et al., 2005). Very recently, a *luxS* deletion mutant of *Actinobacillus*
274 *pleuropneumoniae* was shown to be significantly attenuated in an *in vivo* mouse model (Li et
275 al., 2008). The down-regulation of the apxIIA toxin, a major virulence factor of
276 *Actinobacillus pleuropneumoniae*, may contribute to the virulence attenuation of the *luxS*
277 deletion mutant (Li et al., 2008).

278 There are numerous studies dealing with the role of QS in the pathogenesis of
279 infections with EHEC (e.g. Kendall and Sperandio, 2007), but studies of the importance of
280 QS in infections with *E. coli* in veterinary medicine are scarce. Recently, a *luxS* deletion
281 mutant of a rabbit enteropathogenic *E. coli* (EPEC) strain was found to be impaired in its
282 virulence *in vitro*, but was not attenuated in experimentally infected rabbits (Zhu et al., 2007).

283 Even though QS was initially described and thoroughly examined in several *Vibrio*
284 *spp.*, reports linking the QS mechanisms to virulence in fish are much more limited, both for
285 *Vibrio spp.* (Bruhn et al., 2005; Austin and Zhang, 2006; Nelson et al., 2007; Ye et al., 2008)
286 and non-*Vibrio* fish pathogens (Morohoshi et al., 2004; Bruhn et al., 2005; Atkinson et al.,
287 2006; Bi et al., 2007; Rasch et al., 2007).

288 Quorum sensing has been described in various other veterinary or zoonotic pathogens,
289 such as *Clostridium perfringens* (Ohtani et al., 2002), *Yersinia pseudotuberculosis* (Atkinson
290 et al., 2008) and *Campylobacter jejuni* (Jeon et al., 2005; He et al., 2008), but a clear
291 correlation between these QS mechanisms and virulence is yet to be demonstrated.

292

293

294

295 FUTURE PERSPECTIVES OF QUORUM SENSING IN VETERINARY MEDICINE

296

297 *Quorum sensing signal molecule detection as a diagnostic tool?*

298 Since bacterial quorum sensing has been implicated in the onset of bacterial
299 pathogenicity (Wu et al., 2000; Kendall and Sperandio, 2007), the signal molecules produced
300 by the bacterial pathogens are potential biomarkers for the diagnosis and follow-up of
301 bacterial infections (Kumari et al., 2008). Because the chemical structure of the various signal
302 molecules differ greatly, different sensory systems for detecting these molecules have been
303 described (Kalkum et al., 2003; Steindler and Venturi, 2007; Kumari et al., 2008). Most of the
304 studies dealing with the detection of QS signal molecules, however, focus on the detection of
305 AHLs, since these molecules are involved in the development of chronic bacterial infections
306 with *P. aeruginosa* or *Burkholderia cepacia* in the lungs of cystic fibrosis patients in human
307 medicine (Kumari et al., 2008). Despite the plethora of detection systems, only a fraction can
308 be used to detect QS signal molecules in clinical samples (Kumari et al., 2008).

309 AHLs of various Gram-negative bacterial pathogens have been detected in clinical
310 samples of sputum, mucopurulent secretions of the respiratory tract, lung, liver and spleen
311 homogenates and feces of humans and/or experimentally infected mice (Middleton et al.,
312 2002; Jacobi et al., 2003; Kumari et al., 2006; Kumari et al., 2008). In bronchoalveolar
313 lavages (BALs) from transplanted human lungs, long-chain AHLs were detected, even in
314 lungs that were clinically stable and showed no signs of bacterial infection (Ward et al.,
315 2003). Therefore, the detection of QS signal molecules as a means to detect bacterial
316 infections in a very early stage merits further attention. There are currently no reports of
317 detections of QS signal molecules of veterinary bacterial pathogens in clinical samples of
318 animal host species of veterinary importance.

319

320 *Quorum sensing inhibition as a therapeutic tool?*

321 The concept of QS inhibition, as used by a range of prokaryotic and eukaryotic
322 organisms, is not new. For example, certain *S. aureus* strains produce auto-inducers that
323 stimulate their own QS mechanisms, but have a cross-inhibitory effect on the QS systems of
324 other strains, possibly excluding them from infection or colonization sites (Ji et al., 1997).
325 Certain *Bacillus* sp. harbour the acyl-homoserine lactonase enzyme, which inactivates AHL
326 activity by hydrolysing the lactone bond of AHLs of competing bacterial species (Dong et al.,
327 2001). The fact that certain human cell lines are able to inactivate QS signal molecules
328 suggests that mammalian host defences might have developed interfering QS systems against
329 bacterial infections as well (Chun et al., 2004).

330 Increasing antimicrobial resistance in pathogenic bacteria has created the need for the
331 development of novel therapeutic agents. It is clear that QS is a key regulatory system in the
332 pathogenesis of various bacterial infections. Therefore, the targeting of the QS mechanisms
333 provides a novel strategy for combating bacterial infections (Bjarnsholt and Givskov, 2007).
334 These compounds ideally have no direct effect on bacterial growth, but attenuate bacterial
335 pathogenicity, thereby increasing the susceptibility of the pathogen to the host defences. Even
336 though the macrolides may show potential as QS inhibitors (Tateda et al., 2007), the lack of
337 an effect of “pure” QS inhibitors on bacterial growth could mean a strong advantage relative
338 to the “classic” antimicrobial agents, since the use of these products may not exert a strong
339 selective pressure towards the development of resistance.

340 Very recently, a small molecule, LED209, was discovered using a high-throughput
341 screening which inhibits the QseC mediated activation of virulence gene expression and
342 subsequent *in vivo* virulence of various bacterial pathogens, such as EHEC, *Salmonella*
343 Typhimurium and *Francisella tularensis* (Rasko et al., 2008). Being non-toxic for mammals,

344 having a broad spectrum and lacking a direct effect on bacterial growth, this molecule holds a
345 promising future (Rasko et al., 2008).

346 Inhibition of biofilm formation has been described using QS inhibitors in *P.*
347 *aeruginosa* (Bjarnsholt and Givskov, 2007), *S. aureus* (Wright et al., 2005) and *Salmonella*
348 *Typhimurium* (Janssens et al., 2007).

349 Research aimed at the use of QS inhibitors in veterinary medicine is currently limited
350 to their use in aquaculture. For the fish pathogens *Aeromonas salmonicida* and *Vibrio*
351 (*Listonella*) *anguillarum*, AHL analogues reduced the virulence gene expression and the fish
352 mortality rates, respectively (Rasch et al., 2004; Rasch et al., 2007).

353

354 CONCLUSION

355 Quorum sensing is a widespread phenomenon used by various bacterial pathogens of
356 veterinary importance. Different QS mechanisms make use of diverse signal molecules,
357 leading to intraspecies, interspecies and even interkingdom signalling. Since QS probably
358 plays a role in the pathogenesis of many bacterial infections in animals, the detection of QS
359 signal molecules at early stages of the infection and the use of QS inhibitors for treatment
360 may provide new tools in future veterinary medicine.

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364 REFERENCES

365

366 Atkinson, S., Sockett, R.E., Cámara, M., Williams, P., 2006. Quorum sensing and the lifestyle
367 of *Yersinia*. *Curr. Issues Mol. Biol.* 8, 1-10.

368 Atkinson, S., Chang, C.Y., Patrick, H.L., Buckley, C.M., Wang, Y., Sockett, R.E., Cámara,
369 M., Williams, P., 2008. Functional interplay between the *Yersinia pseudotuberculosis*
370 YpsRI and YtbRI quorum sensing systems modulates swimming motility by
371 controlling expression of flhDC and fliA. *Mol. Microbiol.* 69, 137-151.

372 Austin, B., Zhang, X.H. (2006). *Vibrio harveyi*: a significant pathogen of marine vertebrates
373 and invertebrates. *Lett. Appl. Microbiol.* 43, 119-124.

374 Bearson, B.L., Bearson, S.M., 2007. The role of the QseC quorum-sensing sensor kinase in
375 colonization and norepinephrine-enhanced motility of *Salmonella enterica* serovar
376 Typhimurium. *Microb. Pathog.* 44, 271-278.

377 Bearson, B.L., Bearson, S.M., Uthe, J.J., Dowd, S.E., Houghton, J.O., Lee, I., Toscano, M.J.,
378 Lay, D.C. Jr., 2008. Iron regulated genes of *Salmonella enterica* serovar Typhimurium
379 in response to norepinephrine and the requirement of fepDGC for norepinephrine-
380 enhanced growth. *Microbes Infect.* 10, 807-816.

381 Bi, Z.X., Liu, Y.J., Lu, C.P., 2007. Contribution of AhyR to virulence of *Aeromonas*
382 *hydrophila* J-1. *Res. Vet. Sci.* 83, 150-156.

383 Bjarnsholt, T., Givskov, M., 2007. Quorum-sensing blockade as a strategy for enhancing host
384 defences against bacterial pathogens. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 362,
385 1213-1222.

386 Bohez, L., Ducatelle, R., Pasmans, F., Botteldoorn, N., Haesebrouck, F., Van Immerseel, F.,
387 2006. *Salmonella enterica* serovar Enteritidis colonization of the chicken caecum
388 requires the HilA regulatory protein. *Vet. Microbiol.* 116, 202-210.

389 Boles, B.R., Horswill, A.R., 2008. Agr-mediated dispersal of *Staphylococcus aureus* biofilms.
390 *PLoS Pathog.* 4, e1000052.

391 Booth, M.C., Atkuri, R.V., Nanda, S.K., Iandolo, J.J., Gilmore, M.S., 1995. Accessory gene
392 regulator controls *Staphylococcus aureus* virulence in endophthalmitis. *Invest.*
393 *Ophthalmol. Vis. Sci.* 36, 1828-1836.

394 Boyen, F., Pasmans, F., Van Immerseel, F., Morgan, E., Adriaensen, C., Hernalsteens, J.-P.,
395 Decostere, A., Ducatelle, R., Haesebrouck, F. (2006). *Salmonella* Typhimurium SPI-1

396 genes promote intestinal but not tonsillar colonization in pigs. *Microbes Infect.* 8,
397 2899-2907.

398 Bruhn, J.B., Dalsgaard, I., Nielsen, K.F., Buchholtz, C., Larsen, J.L., Gram, L., 2005. Quorum
399 sensing signal molecules (acylated homoserine lactones) in gram-negative fish
400 pathogenic bacteria. *Dis. Aquat. Organ.* 65, 43-52.

401 Buzzola, F.R., Alvarez, L.P., Tuchscher, L.P., Barbagelata, M.S., Lattar, S.M., Calvinho, L.,
402 Sordelli, D.O., 2007. Differential abilities of capsulated and noncapsulated
403 *Staphylococcus aureus* isolates from diverse agr groups to invade mammary epithelial
404 cells. *Infect. Immun.* 75, 886-891.

405 Cheung, A.L., Eberhardt, K.J., Chung, E., Yeaman, M.R., Sullam, P.M., Ramos, M., Bayer,
406 A.S., 1994. Diminished virulence of a sar-/agr- mutant of *Staphylococcus aureus* in
407 the rabbit model of endocarditis. *J. Clin. Invest.* 94, 1815-1822.

408 Choi, J., Shin, D., Ryu, S., 2007. Implication of quorum sensing in *Salmonella enterica*
409 serovar typhimurium virulence: the *luxS* gene is necessary for expression of genes in
410 pathogenicity island 1. *Infect. Immun.* 75, 4885-4890.

411 Chun, C.K., Ozer, E.A., Welsh, M.J., Zabner, J., Greenberg, E.P., 2004. Inactivation of a
412 *Pseudomonas aeruginosa* quorum-sensing signal by human airway epithelia. *Proc.*
413 *Natl. Acad. Sci. U. S. A.* 101, 3587-3590.

414 Corbeil, L.B., 2007. *Histophilus somni* host-parasite relationships. *Anim. Health Res. Rev.* 8,
415 151-160.

416 Devriese, L.A., Hermans, K., Baele, M., Haesebrouck, F., 2009. *Staphylococcus*
417 *pseudintermedius* versus *Staphylococcus intermedius*. *Vet. Microbiol.* 133, 206-207.

418 Devriese, L.A., Vancanneyt, M., Baele, M., Vaneechoutte, M., De Graef, E., Snauwaert, C.,
419 Cleenwerck, I., Dawyndt, P., Swings, J., Decostere, A., Haesebrouck, F., 2005.
420 *Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals.
421 *Int. J. Syst. Evol. Microbiol.* 55, 1569-1573.

422 Dirix, G., Monsieurs, P., Dombrecht, B., Daniels, R., Marchal, K., Vanderleyden, J.,
423 Michiels, J., 2004. Peptide signal molecules and bacteriocins in Gram-negative
424 bacteria: a genome-wide in silico screening for peptides containing a double-glycine
425 leader sequence and their cognate transporters. *Peptides.* 25, 1425-1440.

426 Dong, Y.H., Wang, L.H., Xu, J.L., Zhang, H.B., Zhang, X.F., Zhang, L.H., 2001. Quenching
427 quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase.
428 *Nature* 411, 813-817.

429 Dufour, P., Jarraud, S., Vandenesch, F., Greenland, T., Novick, R.P., Bes, M., Etienne, J.,
430 Lina, G., 2002. High genetic variability of the *agr* locus in *Staphylococcus* species. *J.*
431 *Bacteriol.* 184, 1180-1186.

432 Dunny, G.M., Brickman, T.J., Dworkin, M., 2008. Multicellular behavior in bacteria:
433 communication, cooperation, competition and cheating. *Bioessays* 30, 296-298.

434 Fuqua, C., Parsek, M.R., Greenberg, E.P., 2001. Regulation of gene expression by cell-to-cell
435 communication: acyl-homoserine lactone quorum sensing. *Annu. Rev. Genet.* 35, 439-
436 468.

437 Gillaspay, A.F., Hickmon, S.G., Skinner, R.A., Thomas, J.R., Nelson, C.L., Smeltzer, M.S.,
438 1995. Role of the accessory gene regulator (*agr*) in pathogenesis of staphylococcal
439 osteomyelitis. *Infect. Immun.* 63, 3373-3380.

440 Girard, G., Bloemberg, G.V., 2008. Central role of quorum sensing in regulating the
441 production of pathogenicity factors in *Pseudomonas aeruginosa*. *Future Microbiol.* 3,
442 97-106.

443 Goerke, C., Kummel, M., Dietz, K., Wolz, C., 2003. Evaluation of intraspecies interference
444 due to *agr* polymorphism in *Staphylococcus aureus* during infection and colonization.
445 *J. Infect. Dis.* 188, 250-256.

446 Haesebrouck, F., Van Immerseel, F., Hermans, K., Martel, A., Ducatelle, R. Pasmans, F.,
447 2007. Biofilms: Betekenis voor de behandeling en bestrijding van bacteriële infecties
448 bij huisdieren. *Vlaams Diergen. Tijds.* 76, 331-336.

449 Hajek, V., 1976. *Staphylococcus intermedius*, a new species isolated from animals, *Int. J.*
450 *Syst. Bacteriol.* 26, 401-408.

451 Hammer, A.S., Pedersen, K., Andersen, T.H., Jørgensen, J.C., Dietz, H.H., 2003. Comparison
452 of *Pseudomonas aeruginosa* isolates from mink by serotyping and pulsed-field gel
453 electrophoresis. *Vet. Microbiol.* 94, 237-243.

454 Hardie, K.R., Heurlier, K., 2008. Establishing bacterial communities by 'word of mouth':
455 LuxS and autoinducer 2 in biofilm development. *Nat. Rev. Microbiol.* 6, 635-643.

456 He, Y., Frye, J.G., Strobaugh, T.P., Chen, C.Y., 2008. Analysis of AI-2/LuxS-dependent
457 transcription in *Campylobacter jejuni* strain 81-176. *Foodborne Pathog. Dis.* 5, 399-
458 415.

459 Irie, Y., Parsek, M.R., 2008. Quorum sensing and microbial biofilms. *Curr. Top. Microbiol.*
460 *Immunol.* 322, 67-84.

461 Jacobi, C.A., Bach, A., Eberl, L., Steidle, A., Heesemann, J., 2003. Detection of N-(3-
462 oxohexanoyl)-L-homoserine lactone in mice infected with *Yersinia enterocolitica*
463 serotype O8. *Infect. Immun.* 71, 6624-6626.

464 Janssens, J.C., Steenackers, H., Metzger, K., Daniels, R., Ptacek, D., Verhoeven, T., Hermans,
465 K., Vanderleyden, J., De Vos, D.E., De Keersmaecker, S.C., 2007. Interference with
466 the quorum sensing systems of *Salmonella enterica* serovar typhimurium: possibilities
467 and implications. *Commun. Agric. Appl. Biol. Sci.* 72, 35-39.

468 Jeon, B., Itoh, K., Ryu, S., 2005. Promoter analysis of cytolethal distending toxin genes
469 (*cdtA*, *B*, and *C*) and effect of a *luxS* mutation on CDT production in *Campylobacter*
470 *jejuni*. *Microbiol. Immunol.* 49, 599-603.

471 Ji, G., Beavis, R., Novick, R.P., 1997. Bacterial interference caused by autoinducing peptide
472 variants. *Science* 276, 2027-2030.

473 Kalkum, M., Lyon, G.J., Chait, B.T., 2003. Detection of secreted peptides by using
474 hypothesis-driven multistage mass spectrometry. *Proc. Natl. Acad. Sci. U. S. A.* 100,
475 2795-2800.

476 Karch, H., Tarr, P.I., Bielaszewska, M., 2005. Enterohaemorrhagic *Escherichia coli* in human
477 medicine. *Int. J. Med. Microbiol.* 295, 405-418.

478 Keller, R.L., Hendrix, D.V., 2005. Bacterial isolates and antimicrobial susceptibilities in
479 equine bacterial ulcerative keratitis (1993--2004). *Equine Vet. J.* 37, 207-211.

480 Kendall, M.M., Sperandio, V., 2007. Quorum sensing by enteric pathogens. *Curr. Opin.*
481 *Gastroenterol.* 23, 10-15.

482 Kingsford, N.M., Raadsma, H.W., 1997. The occurrence of *Pseudomonas aeruginosa* in
483 fleece washings from sheep affected and unaffected with fleece rot. *Vet. Microbiol.* 54,
484 275-285.

485 Kleerebezem, M., 2004. Quorum sensing control of lantibiotic production; nisin and subtilin
486 autoregulate their own biosynthesis. *Peptides* 25, 1405-1414.

487 Kumari, A., Pasini, P., Daunert, S., 2008. Detection of bacterial quorum sensing N-acyl
488 homoserine lactones in clinical samples. *Anal. Bioanal. Chem.* 391, 1619-1627.

489 Kumari, A., Pasini, P., Deo, S.K., Flomenhoft, D., Shashidhar, H., Daunert, S., 2006.
490 Biosensing systems for the detection of bacterial quorum signaling molecules. *Anal.*
491 *Chem.* 78, 7603-7609.

492 Lazazzera, B.A., 2000. Quorum sensing and starvation: signals for entry into stationary phase.
493 *Curr. Opin. Microbiol.* 3, 177-182.

494 Li, L., Zhou, R., Li, T., Kang, M., Wan, Y., Xu, Z., Chen, H., 2008. Enhanced biofilm
495 formation and reduced virulence of *Actinobacillus pleuropneumoniae luxS* mutant.
496 *Microb. Pathog.* 45, 192-200.

497 Lyon, G.J., Novick, R.P., 2004. Peptide signaling in *Staphylococcus aureus* and other Gram-
498 positive bacteria. *Peptides.* 25, 1389-1403.

499 Malott, R.J., Lo, R.Y., 2002. Studies on the production of quorum-sensing signal molecules in
500 *Mannheimia haemolytica* A1 and other Pasteurellaceae species. *FEMS Microbiol.*
501 *Lett.* 206, 25-30.

502 McLennan, M.W., Kelly, W.R., O'Boyle, D., 1997. *Pseudomonas* mastitis in a dairy herd.
503 *Aust. Vet. J.* 75, 790-792.

504 Melchior, M.B., van Osch, M.H.J., Graat, R.M., van Duijkeren, E., Mevius, D.J., Nielen, M.,
505 Gaastra, W., Fink-Gremmels, J. (2009). Biofilm formation and genotyping of
506 *Staphylococcus aureus* bovine mastitis isolates: evidence for lack of penicillin-
507 resistance in *Agr*- type II strains., *Vet. Microbiol.*, in press.
508 doi:10.1016/j.vetmic.2008.12.004

509 Michael, B., Smith, J.N., Swift, S., Heffron, F., Ahmer, B.M., 2001. SdiA of *Salmonella*
510 *enterica* is a LuxR homolog that detects mixed microbial communities. *J. Bacteriol.*
511 183, 5733-5742.

512 Middleton, B., Rodgers, H.C., Cámara, M., Knox, A.J., Williams, P., Hardman, A., 2002.
513 Direct detection of N-acylhomoserine lactones in cystic fibrosis sputum. *FEMS*
514 *Microbiol. Lett.* 207, 1-7.

515 Morgan, E., Campbell, J.D., Rowe, S.C., Bispham, J., Stevens, M.P., Bowen, A.J., Barrow,
516 P.A., Maskell, D.J., Wallis, T.S. (2004). Identification of host-specific colonization
517 factors of *Salmonella enterica* serovar Typhimurium. *Mol. Microbiol.* 54, 994-1010.

518 Moré, M.I., Finger, L.D., Stryker, J.L., Fuqua, C., Eberhard, A., Winans, S.C., 1996.
519 Enzymatic synthesis of a quorum-sensing autoinducer through use of defined
520 substrates. *Science.* 272, 1655-1658.

521 Morohoshi, T., Inaba, T., Kato, N., Kanai, K., Ikeda, T., 2004. Identification of quorum-
522 sensing signal molecules and the LuxRI homologs in fish pathogen *Edwardsiella*
523 *tarda*. *J. Biosci. Bioeng.* 98, 274-281.

524 Moxley, R.A., 2004. *Escherichia coli* 0157:H7: an update on intestinal colonization and
525 virulence mechanisms. *Anim. Health. Res. Rev.* 5, 15-33.

526 Nealson, K.H., Platt, T., Hastings, J.W., 1970. Cellular control of the synthesis and activity of
527 the bacterial luminescent system. *J. Bacteriol.* 104, 313-322.

528 Neiditch, M.B., Federle, M.J., Miller, S.T., Bassler, B.L., Hughson, F.M., 2005. Regulation of
529 LuxPQ receptor activity by the quorum-sensing signal autoinducer-2. *Mol. Cell.* 18,
530 507-518.

531 Nelson, E.J., Tunsjø, H.S., Fidopiastis, P.M., Sørum, H., Ruby, E.G., 2007. A novel lux
532 operon in the cryptically bioluminescent fish pathogen *Vibrio salmonicida* is
533 associated with virulence. *Appl. Environ. Microbiol.* 73, 1825-1833.

534 Novick, R.P., 2003. Autoinduction and signal transduction in the regulation of staphylococcal
535 virulence. *Mol. Microbiol.* 48, 1429-1449.

536 Novick, R.P., Geisinger, E., 2008. Quorum sensing in staphylococci. *Annu. Rev. Genet.* 42,
537 541-564.

538 Ohtani, K., Hayashi, H., Shimizu, T., 2002. The *luxS* gene is involved in cell-cell signalling
539 for toxin production in *Clostridium perfringens*. *Mol. Microbiol.* 44, 171-179.

540 Rajamani, S., Zhu, J., Pei, D., Sayre, R., 2007. A LuxP-FRET-based reporter for the detection
541 and quantification of AI-2 bacterial quorum-sensing signal compounds. *Biochemistry.*
542 46, 3990-3997.

543 Rasch, M., Buch, C., Austin, B., Slierendrecht, W.J., Ekmann, K.S., Larsen, J.L., Johansen,
544 C., Riedel, K., Eberl, L., Givskov, M., Gram, L., 2004. An inhibitor of bacterial
545 quorum sensing reduces mortalities caused by Vibriosis in rainbow trout
546 (*Oncorhynchus mykiss*, Walbaum). *Syst. Appl. Microbiol.* 27, 350-359.

547 Rasch, M., Kastbjerg, V.G., Bruhn, J.B., Dalsgaard, I., Givskov, M., Gram, L., 2007. Quorum
548 sensing signals are produced by *Aeromonas salmonicida* and quorum sensing
549 inhibitors can reduce production of a potential virulence factor. *Dis. Aquat. Organ.* 78,
550 105-113.

551 Rasko, D.A., Moreira, C.G., Li, de R., Reading, N.C., Ritchie, J.M., Waldor, M.K., Williams,
552 N., Taussig, R., Wei, S., Roth, M., Hughes, D.T., Huntley, J.F., Fina, M.W., Falck,
553 J.R., Sperandio, V., 2008. Targeting QseC signaling and virulence for antibiotic
554 development. *Science* 321, 1078-1080.

555 Rezzonico, F., Duffy, B., 2008. Lack of genomic evidence of AI-2 receptors suggests a non-
556 quorum sensing role for luxS in most bacteria. *BMC Microbiol.* 8, 154.

557 Rubin, J., Walker, R.D., Blickenstaff, K., Bodeis-Jones, S., Zhao, S., 2008. Antimicrobial
558 resistance and genetic characterization of fluoroquinolone resistance of *Pseudomonas*
559 *aeruginosa* isolated from canine infections. *Vet. Microbiol.* 131, 164-172.

560 Salmond, G.P.C., Bycroft, B.W., Stewart, G.S.A.B., Williams, P., 1995. The bacterial
561 'enigma': cracking the code of cell-cell communication. *Mol. Microbiol.* 16, 615-624.

562 Schauder, S., Shokat, K., Surette, M.G., Bassler, B.L., 2001. The LuxS family of bacterial
563 autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol. Microbiol.*
564 41, 463-476.

565 Smith, D., Wang, J.H., Swatton, J.E., Davenport, P., Price, B., Mikkelsen, H., Stickland, H.,
566 Nishikawa, K., Gardiol, N., Spring, D.R., Welch, M., 2006. Variations on a theme:
567 diverse N-acyl homoserine lactone-mediated quorum sensing mechanisms in gram-
568 negative bacteria. *Sci. Prog.* 89, 167-211.

569 Smith, J.N., Dyszel, J.L., Soares, J.A., Ellermeier, C.D., Altier, C., Lawhon, S.D., Adams,
570 L.G., Konjufca, V., Curtiss, R. 3rd, Slauch, J.M., Ahmer, B.M., 2008. SdiA, an N-
571 acylhomoserine lactone receptor, becomes active during the transit of *Salmonella*
572 *enterica* through the gastrointestinal tract of turtles. *PLoS ONE* 3, e2826.

573 Surette, M.G., Miller, M.B., Bassler, B.L., 1999. Quorum sensing in *Escherichia coli*,
574 *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for
575 autoinducer production. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1639-1644.

576 Tateda, K., Ishii, Y., Kimura, S., Horikawa, M., Miyairi, S., Yamaguchi, K., 2007.
577 Suppression of *Pseudomonas aeruginosa* quorum-sensing systems by macrolides: a
578 promising strategy or an oriental mystery? *J. Infect. Chemother.* 13, 357-367.

579 Traber, K.E., Lee, E., Benson, S., Corrigan, R., Cantera, M., Shopsin, B., Novick, R.P., 2008.
580 Agr function in clinical *Staphylococcus aureus* isolates. *Microbiology-(UK)* 154,
581 2265-2274.

582 Tron, E.A., Wilke, H.L., Petermann, S.R., Rust, L., 2004. *Pseudomonas aeruginosa* from
583 canine otitis externa exhibit a quorum sensing deficiency. *Vet. Microbiol.* 99, 121-129.

584 Vancraeynest, D., Haesebrouck, F., Deplano, A., Denis, O., Godard, C., Wildemauwe, C.,
585 Hermans, K., 2006. International dissemination of a high virulence rabbit
586 *Staphylococcus aureus* clone. *J. Vet. Med. B Infect. Dis. Vet. Public Health.* 53, 418-
587 422.

588 van der Vinne, A.N., Lo, R.Y., Shewen, P.E., 2005. Construction and analysis of a
589 *Mannheimia haemolytica* A1 *luxS* mutant. *Vet. Microbiol.* 110, 53-66.

590 Vautor, E., Magnone, V., Rios, G., Le Brigand, K., Bergonier, D., Lina, G., Meugnier, H.,
591 Barbry, P., Thiéry, R., Pépin, M., 2008. Genetic differences among *Staphylococcus*
592 *aureus* isolates from dairy ruminant species: A single-dye DNA microarray approach.
593 *Vet. Microbiol.* [Epub ahead of print]: doi:10.1016/j.vetmic.2008.06.006.

594 Vendeville, A., Winzer, K., Heurlier, K., Tang, C.M., Hardie, K.R., 2005. Making 'sense' of
595 metabolism: autoinducer-2, LuxS and pathogenic bacteria. *Nat. Rev. Microbiol.* 3,
596 383-396.

597 Walters, M., Sperandio, V., 2006. Quorum sensing in *Escherichia coli* and *Salmonella*. *Int. J.*
598 *Med. Microbiol.* 296, 125-131.

599 Ward, C., Cámara, M., Forrest, I., Rutherford, R., Pritchard, G., Daykin, M., Hardman, A., de
600 Soyza, A., Fisher, A.J., Williams, P., Corris, P.A., 2003. Preliminary findings of
601 quorum signal molecules in clinically stable lung allograft recipients. *Thorax.* 58, 444-
602 446.

603 Waters, C.M., Bassler, B.L., 2005. Quorum sensing: cell-to-cell communication in bacteria.
604 *Annu. Rev. Cell. Dev. Biol.* 21, 319-346.

605 Weese, J.S., 2008. A review of multidrug resistant surgical site infections. *Vet. Comp.*
606 *Orthop. Traumatol.* 21, 1-7.

607 Whiteley, M., Lee, K.M., Greenberg, E.P., 1999. Identification of genes controlled by quorum
608 sensing in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U. S. A.* 96, 13904-13909.

609 Wright, J.S. 3rd, Jin, R., Novick, R.P., 2005. Transient interference with staphylococcal
610 quorum sensing blocks abscess formation. *Proc. Natl. Acad. Sci. U. S. A.* 102, 1691-
611 1696.

612 Wu, H., Song, Z., Hentzer, M., Andersen, J.B., Heydorn, A., Mathee, K., Moser, C., Eberl, L.,
613 Molin, S., Høiby, N., Givskov, M., 2000. Detection of N-acylhomoserine lactones in
614 lung tissues of mice infected with *Pseudomonas aeruginosa*. *Microbiology-(UK)* 146,
615 2481-2493.

616 Xavier, KB, Bassler, BL., 2003. LuxS quorum sensing: more than just a numbers game. *Curr.*
617 *Opin. Microbiol.* 6, 191-197.

618 Ye, J., Ma, Y., Liu, Q., Zhao, D.L., Wang, Q.Y., Zhang, Y.X., 2008. Regulation of *Vibrio*
619 *alginolyticus* virulence by the LuxS quorum-sensing system. *J. Fish Dis.* 31, 161-169.

620 Zhu, C., Feng, S., Sperandio, V., Yang, Z., Thate, T.E., Kaper, J.B., Boedeker, E.C., 2007.
621 The possible influence of LuxS in the *in vivo* virulence of rabbit enteropathogenic
622 *Escherichia coli*. *Vet. Microbiol.* 125, 313-322.

623

624

625 **Figure legend**

626

627 **Figure 1:** The principle of quorum sensing is depicted. In situations where the bacterial
628 density is low (left), the signal molecule concentration is also low and does not exceed the
629 quorum sensing threshold. In case the bacterial density is high (right), the signal molecule
630 concentration is also high and the quorum sensing threshold is exceeded. As a result, the signal
631 molecules are bound to their receptors on the bacterial cells and the gene expression of the
632 total population is changed and synchronised.

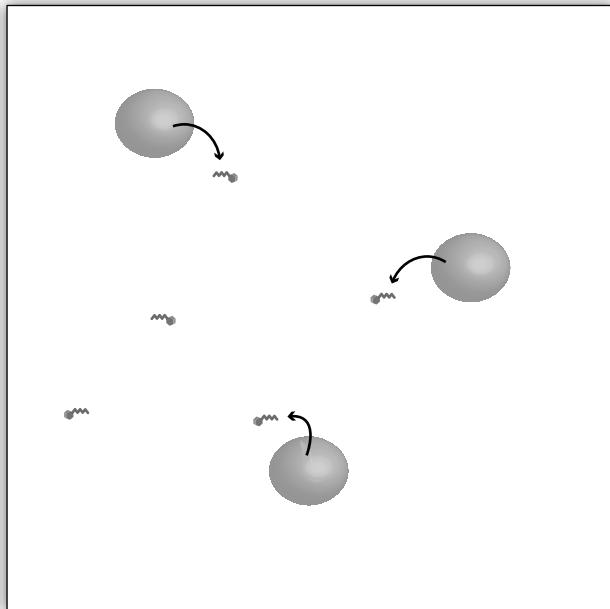
633

634 **Figure 2:** Four simplified models of the described quorum sensing mechanisms.

635

Figure 1

Low bacterial density
Low signal molecule concentration



High bacterial density
High signal molecule concentration

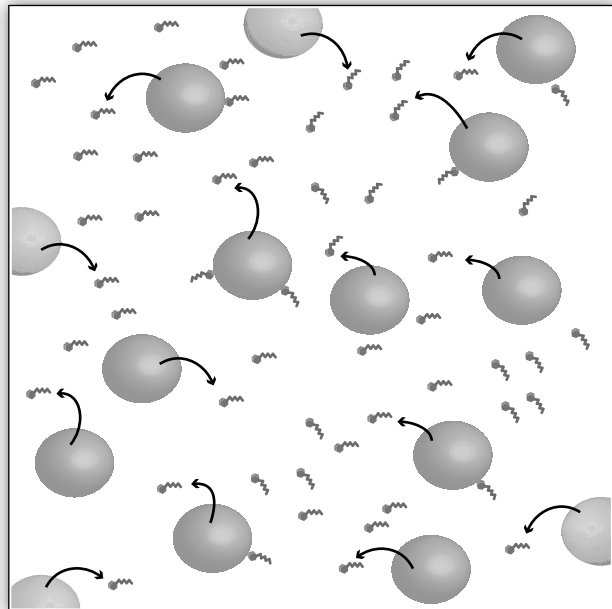
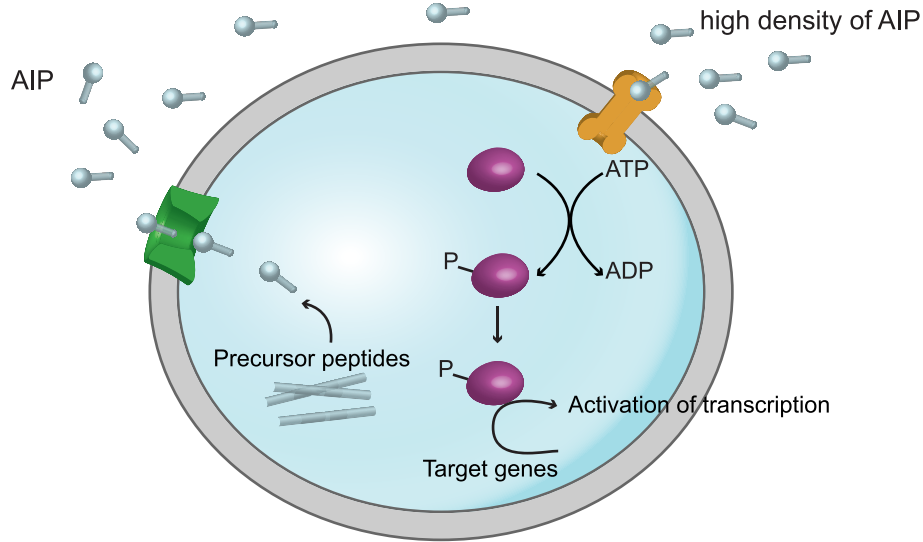


Figure 2

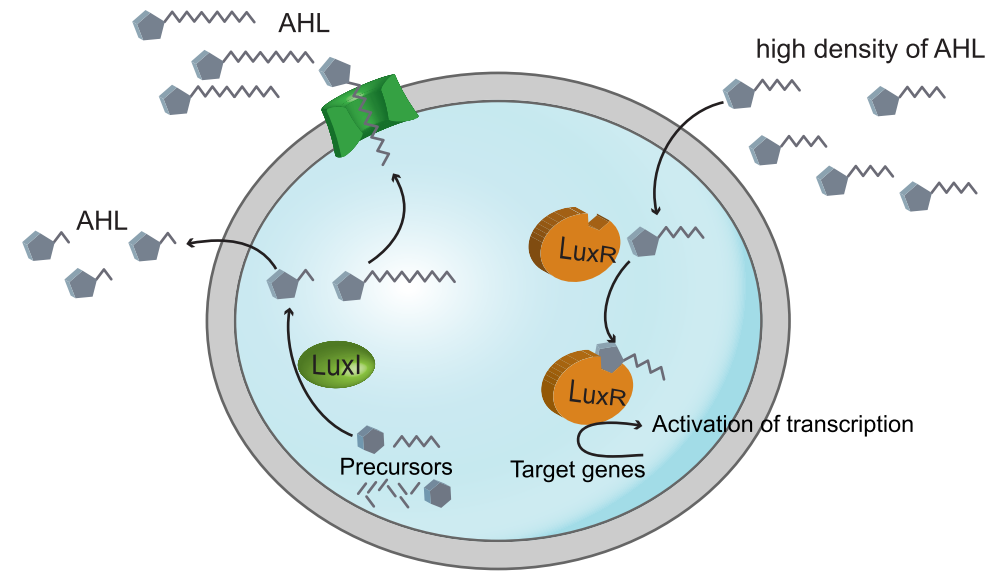
MECHANISM 1

Quorum sensing in mainly Gr⁺ bacteria: autoinducing polypeptides (AIP)



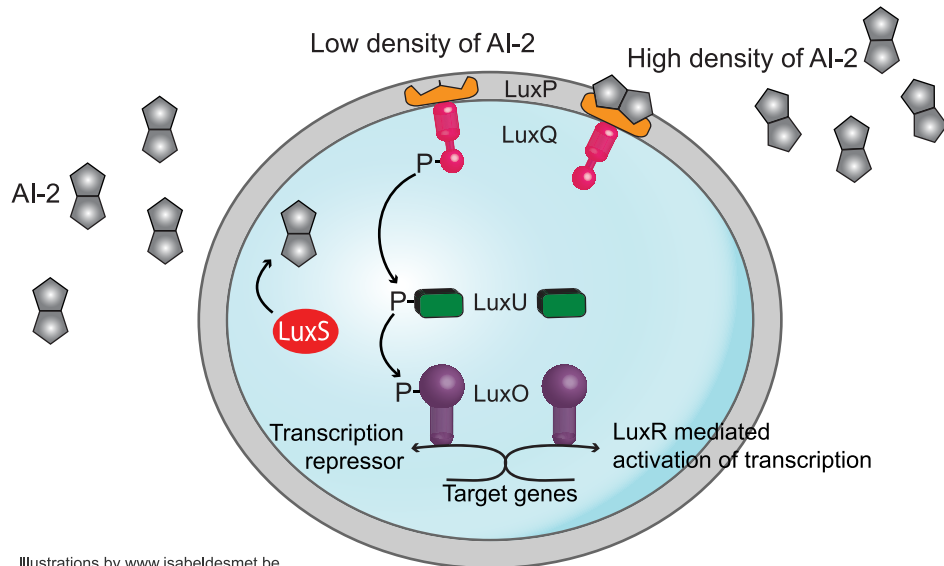
MECHANISM 2

Quorum sensing in Gr⁻ bacteria: N-acylhomoserine lactones (AHL / AI-1)



MECHANISM 3

Quorum sensing in both Gr⁺ and Gr⁻ bacteria: autoinducer 2 (AI-2)



MECHANISM 4

Quorum sensing beyond bacterial borders: autoinducer 3 (AI-3)

