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## Position Paper

### A proposal of clinical breakpoints for amoxicillin applicable to porcine respiratory tract pathogens

Stefan Schwarz,<sup>a,\*</sup> Alexander Böttner,<sup>b</sup> H. Mohamed Hafez,<sup>c</sup> Luc Goosens,<sup>d</sup> Katrin Hartmann,<sup>e</sup> Martin Kaske,<sup>f</sup> Corinna Kehrenberg,<sup>a</sup> Manfred Kietzmann,<sup>g</sup> Dieter Klarmann,<sup>h</sup> Günter Klein,<sup>i</sup> Peter Krabisch,<sup>j</sup> Gabriele Luhofer,<sup>k</sup> Angelika Richter,<sup>l</sup> Bianka Schulz,<sup>e</sup> Claudia Sigge,<sup>m</sup> Karl-Heinz Waldmann,<sup>n</sup> Jürgen Wallmann,<sup>o</sup> and Christiane Werckenthin<sup>p</sup>

<sup>a</sup> Institut für Tierzucht, Bundesforschungsanstalt für Landwirtschaft (FAL), Neustadt-Mariensee,

<sup>b</sup> Intervet Innovation GmbH, Schwabenheim,

<sup>c</sup> Institut für Geflügelkrankheiten, Freie Universität Berlin,

<sup>d</sup> Pfizer GmbH, Karlsruhe,

<sup>e</sup> Medizinische Kleintierklinik, Ludwig-Maximilians-Universität München,

<sup>f</sup> Institut für Physiologie, Wissenschaftszentrum Weihenstephan, Freising-Weihenstephan,

<sup>g</sup> Institut für Pharmakologie, Toxikologie and Pharmazie, Tierärztliche Hochschule Hannover,

<sup>h</sup> Veterinärinstitut Oldenburg, LAVES,

<sup>i</sup> Institut für Lebensmittelqualität and -sicherheit, Tierärztliche Hochschule Hannover,

<sup>j</sup> TGD Bayern, Poing,

<sup>k</sup> Landesuntersuchungsamt Rheinland-Pfalz, Institut für Lebensmittel tierischer Herkunft, Koblenz,

<sup>l</sup> Institut für Pharmakologie and Toxikologie, Freie Universität Berlin,

<sup>m</sup> Bundesverband für Tiergesundheit (BfT), Bonn,

<sup>n</sup> Klinik für kleine Klautiere, Tierärztliche Hochschule Hannover,

<sup>o</sup> Bundesamt für Verbraucherschutz and Lebensmittelsicherheit (BVL), Berlin,

<sup>p</sup> Institut für Medizinische Mikrobiologie, Infektions- and Seuchenmedizin, Ludwig-Maximilians-Universität München.

short title: clinical breakpoints for amoxicillin

32

33 \* Corresponding author. Tel: +49-5034-871-241, Fax: +49-5034-871-246.

34 *E-mail address:* [stefan.schwarz@fal.de](mailto:stefan.schwarz@fal.de) (S. Schwarz)

35

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35 **Abstract**

36

37 In the present position paper, an attempt was made to establish clinical  
38 breakpoints of amoxicillin to classify porcine respiratory tract pathogens as  
39 susceptible, intermediate or resistant based on their minimum inhibitory  
40 concentrations of amoxicillin. For this, a thorough review of the published  
41 literature with regard to swine-specific pharmacological data (including dosages  
42 of amoxicillin applied and routes of administration used), clinical efficacy, and *in*  
43 *vitro* susceptibility of the target pathogens was performed. Based on the  
44 comparative analysis of the results, the working group “Antibiotic Resistance” of  
45 the German Society for Veterinary Medicine (DVG) proposed to classify porcine  
46 respiratory tract pathogens that show MIC values of amoxicillin of  $\leq 0.5 \mu\text{g/ml}$  as  
47 “susceptible”, those with MICs of  $1 \mu\text{g/ml}$  as “intermediate”, and those with MICs  
48 of  $\geq 2 \mu\text{g/ml}$  as “resistant”.

49

50

51

52 **Key words:** antibiotic therapy, breakpoints, swine, respiratory diseases, amoxicillin

53

## 53 1. Introduction

54

55 According to prudent use guidelines, the *in-vitro* susceptibility of bacteria against  
56 antimicrobial agents of bacteria, which are considered to be causative for a specific  
57 disease condition, should be determined prior to therapy. This approach shall ensure  
58 that only those substances are applied to which the causative bacterial pathogens  
59 exhibit susceptibility under *in-vitro* conditions and, therefore, are likely to result in a  
60 successful treatment. The classification of the tested bacteria as either “susceptible”,  
61 “intermediate”, or “resistant” is based on breakpoints. Currently, two distinct groups of  
62 breakpoints are used: microbiological breakpoints (also referred to as epidemiological  
63 cut-off values) and clinical breakpoints (Bywater et al., 2006). Microbiological  
64 breakpoints differentiate between subpopulations which exhibit different levels of  
65 susceptibility to a specific antimicrobial agent. In bimodal distributions of MIC values, the  
66 microbiological breakpoints separate a presumably susceptible subpopulation from a  
67 presumably resistant subpopulation (Bywater et al., 2006). However, microbiological  
68 breakpoints do not consider pharmacological aspects of the antimicrobial agents  
69 administered. In contrast, the concentration of the antimicrobial agents which can be  
70 achieved by the recommended dosing at the site of infection is a key parameter for the  
71 determination of clinical breakpoints (Bywater et al., 2006). Other aspects relevant for  
72 the determination of clinical breakpoints are the minimum inhibitory concentration of the  
73 causative pathogens as well as pharmacokinetic and toxicological aspects. The  
74 complexity of parameters involved in the determination of clinical breakpoints shows that  
75 clinical breakpoints do not represent values that can be directly measured. Instead, they  
76 have to be derived from the results of extensive studies which take into account

77 microbiological, pharmacological and clinical aspects (Böttner et al., 2000). This has  
78 previously been shown in detail for the establishment of clinical breakpoints for tilmicosin  
79 (Shryock et al., 1996) and ceftiofur (Burton et al., 1996).

80 Approved clinical breakpoints are an important criterion in the decision for or  
81 against a specific antimicrobial agent in therapeutic interventions (Woolcock, 1993; DIN,  
82 1992; NCCLS, 2002b). However, it should be noted that clinical breakpoints do not  
83 guarantee in every case that the treatment will be successful, but they help to minimize  
84 the risk of therapy failures (Richter et al., 2006). While clinical breakpoints are in  
85 principle established for an antimicrobial agent, they initially apply only to the original  
86 pharmaceutical product used and are not necessarily valid for the respective generic  
87 substances.

88 Due to the lack of veterinary-specific data, the classification of veterinary  
89 pathogens as susceptible or resistant is often based on breakpoints adopted from  
90 human medicine (NCCLS, 2002a; 2004). This applies particularly to older antibiotics that  
91 have been in veterinary use for several decades, such as tetracyclines, ampicillin, or  
92 amoxicillin. Since the spectrum of bacterial pathogens as well as the distribution and  
93 metabolism of antimicrobial agents differ distinctly in humans and animals, the non-  
94 verified adoption of breakpoints from human medicine for use in veterinary medicine is  
95 scientifically questionable.

96 This position paper represents an attempt to establish veterinary-specific clinical  
97 breakpoints for amoxicillin based on data available in the published literature. Amoxicillin  
98 is a member of the semi-synthetic aminopenicillin group of antibiotics which exhibit a  
99 broad spectrum of activity against Gram-positive and Gram-negative bacteria and  
100 displays a bactericidal mode of action. Amoxicillin is used extensively in the treatment of

101 bacterial infections of animals and is approved in a variety of formulations for use in  
102 cattle, pigs, poultry, horses, dogs and cats. For pigs, aqueous and oily preparations for  
103 parenteral administration (intravenous, subcutaneous, and intramuscular) are world-  
104 wide approved. Water-soluble powders are also available for oral medication either via  
105 feed or drinking water. Our attempt to deduce a clinical breakpoint for amoxicillin which  
106 shall be applicable to porcine respiratory tract pathogens is based on known data of the  
107 pharmacokinetics of amoxicillin in swine, the results of clinical efficacy studies, and  
108 available data on the *in-vitro* susceptibility of pathogens causing porcine respiratory tract  
109 infections, such as *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*,  
110 *Streptococcus suis*, *Bordetella bronchiseptica*, and *Haemophilus parasuis*.

111

112

## 113 **2. Pharmacokinetics of amoxicillin in the pig**

114

115 There are several studies which investigated the pharmacokinetic parameters of  
116 amoxicillin in pigs. However, all these studies varied in either the dosages used, the  
117 route of administration of amoxicillin, the health status of the animals, or other  
118 parameters. Moreover, not all the studies listed determined the same parameters by the  
119 same methodology. This situation makes it difficult to compare the results obtained in  
120 the different studies.

121

### 122 **2.1 Key studies**

123

124 A comparative overview of the pharmacokinetics of amoxicillin in the pig after  
125 parenteral and oral administration is presented in Tables 1a-b. The pharmacokinetic



126 parameters of amoxicillin in various formulations and dosages was studied  
127 systematically by Agero and Friis (1998a). To determine its bioavailability, amoxicillin  
128 was administered in different formulations to separate groups of healthy pigs by  
129 intramuscular (i.m.) or oral (p.o.) route in comparison with intravenous (i.v.)  
130 administration. For the i.v. administration, the sodium salt of amoxicillin was used at a  
131 dosage of 8.6 mg/kg body weight. An amoxicillin suspension (trihydrate salt) was  
132 administered in the conventional formulation (Clamoxyl ®) and in the long-acting  
133 formulation (Clamoxyl LA ®) at a dosage of 14.7 mg/kg i.m., respectively. A water-  
134 soluble amoxicillin preparation (Clamoxyl Soluble Powder®) was administered to the  
135 pigs (bodyweight 30–50 kg) by gavage, once on an empty stomach (trial 1) and once  
136 just before feeding (trial 2), at a dosage of 10.1 mg/kg p.o..

137 After **i.v. administration**, the active substance was excreted rapidly. The mean  
138 residence time (MRT) in the serum was 1.2–1.5 h and the volume of distribution at  
139 steady-state ( $V_{ss}$ ) was 0.63–0.55 l/kg. The area under the curve (AUC) values and the  
140 elimination half-life ( $t_{1/2}$  1.8 ± 0.3 h vs. 3.6 ± 0.4 h) differed significantly after i.v. injections  
141 in the two experiments described by Agero and Friis (1998a; see Table 1). Studies  
142 done by Martinez-Larranaga et al. (2004) with i.v. administration of 20 mg/kg amoxicillin  
143 revealed different results as compared to those of Agero and Friis (1998a). In  
144 particular, the AUC, MRT and the  $V_{ss}$  differed (Table 1).

145 After **i.m. administration** of the conventional oily suspension, the active substance  
146 reached its maximum serum concentration ( $C_{max}$ ) of 5.1 µg/ml after 2 h. The MRT was  
147 8.8 h and the bioavailability 83% (Agero and Friis, 1998a; Table 1). The long-acting  
148 formulation of amoxicillin was absorbed more slowly from the injection site, which is  
149 reflected by the longer MRT and by the lower  $C_{max}$ . The first concentration peak of 1.7

150  $\mu\text{g/ml}$  was reached after 1.3 h and the second peak of 0.8  $\mu\text{g/ml}$ , after 6.6 h. The  
151 calculated bioavailability after i.m. injections was 83% or in the case of the long acting  
152 form 126% (Agero and Friis, 1998a).

153 Absorption of amoxicillin after **oral administration** was slower and incomplete,  
154 especially in fed pigs (Table 2), as shown by trial 2 in the studies of Agero and Friis  
155 (1998a). The  $C_{\text{max}}$  value of 1.6  $\mu\text{g/ml}$  was seen after 1.9 h in fasted pigs (trial 1), while a  
156 lower peak concentration of 0.8  $\mu\text{g/ml}$  was reached after 3.6 h in fed pigs (Agero and  
157 Friis, 1998a). Oral bioavailability was only 31% in fasted animals and 28% in fed  
158 animals. However, the observed differences in bioavailability, the  $C_{\text{max}}$  and the time to  
159 maximum serum concentration ( $t_{\text{max}}$ ) were not statistically significant. Agero et al.  
160 (1998) conducted further studies in a herd of 201 pigs. The animals received amoxicillin  
161 at a dosage of 23 mg/kg on 5 consecutive days via **drinking water**. The concentration  
162 of amoxicillin in the blood serum was measured in 10 randomly selected pigs and varied  
163 between 0.5  $\mu\text{g/ml}$  and of 1.3  $\mu\text{g/ml}$ .

164

## 165 2.2 Other studies

166  
167 Agero and Friis (1998b) also conducted studies on the concentration and  
168 distribution of amoxicillin in tissues of healthy pigs. The peak concentration following i.v.  
169 administration of amoxicillin at 8.6 mg/kg was reached after 1 h in the lungs (mucosa:  
170 3.02  $\mu\text{g/ml}$ , tissue: 2.97  $\mu\text{g/ml}$ , and bronchial secretion: 2.1  $\mu\text{g/ml}$ ). Clearance (Cl) of  
171 amoxicillin from the lungs occurred much slower than from the plasma. The results were  
172 confirmed in a further study in pigs suffering from an infection with *A. pleuropneumoniae*  
173 (Agero and Friis, 1998c). The pharmacokinetic parameters did not differ significantly  
174 between healthy and diseased animals after a single i.v. dose of 8.6 mg/kg amoxicillin.

175 However, the concentrations of the active substance were slightly higher in bronchial  
176 secretions (2.29  $\mu\text{g/ml}$ ) of acutely diseased animals and lower in pneumonic lung tissue  
177 (1.70  $\mu\text{g/ml}$ ). The  $C_{\text{max}}$  was reached after 2 h. The coefficient of distribution, measured  
178 from the AUC between tissue and plasma, was 0.53 for bronchial secretion and 0.44 for  
179 both diseased and healthy lung tissue.

180 Sumano et al. (2004) described the bioavailability of three different amoxicillin  
181 premix formulations in a comparative study which differed in the coating of the active  
182 ingredient amoxicillin to protect it from degradation during feed manufacture and storage  
183 conditions. The premixes were administered orally by gavage mixed to feed with  
184 gelatine in order to achieve a dose of 20 mg/kg body weight. As indicated by the higher  
185 AUC, the oral bioavailability of a special amoxicillin formulation, produced by a coating  
186 procedure, was significantly higher when compared with two conventional amoxicillin  
187 products. The  $t_{\text{max}}$  values corresponded well with the results of Agero and Friis (1998a).  
188 However, Sumano et al. (2004) found significant differences between the formulations in  
189 the AUC and in the  $C_{\text{max}}$  values. Interestingly, the  $C_{\text{max}}$  values and the AUC of the  
190 conventional formulations were comparable to the results of Agero and Friis (1998a)  
191 although the administered dose was twice as high at 20 mg/kg. The effect of the  
192 formulation on the oral bioavailability of amoxicillin was also described by Anfossi et al.  
193 (2002), showing that the relative bioavailability can be increased by up to 50% with  
194 specific amoxicillin formulations.

195 Martinez-Larranga et al. (2004) studied the pharmacokinetic parameters of  
196 amoxicillin after single i.v. and oral doses of 20 mg/kg bw administered at five  
197 consecutive days in a cross-over trial in pigs of approximately 80 kg body weight. The  
198  $C_{\text{max}}$  values measured in serum after oral administration were distinctly higher than in

199 the studies of Agerso and Friis (1998a, 1998b) and Sumano et al. (2004), namely 7.4  
200  $\mu\text{g/ml}$  compared with 0.8  $\mu\text{g/ml}$  and 1.6  $\mu\text{g/ml}$ , respectively. Oral bioavailability of 41%  
201 was also considerably higher than the values of 28% - 31% recorded by Agerso and  
202 Friis (1998a, b) (Table 3).

203 Anadon et al. (2000) determined the pharmacokinetic parameters of amoxicillin  
204 after a single oral medication of fasting pigs at a dosage of 20 mg/kg. Therapeutically  
205 relevant serum levels ( $>0.3 \mu\text{g/ml}$ ) were reached after ca. 30 min and persisted for up to  
206 12 h after administration of the drug. The pharmacokinetic parameters were comparable  
207 to those measured by Martinez-Larranga et al. (2004).

208 Hernandez et al. (2005) determined the bioavailability of an oral amoxicillin  
209 formulation after a single medication at 15 mg/kg. Compared with i.v. injections, the oral  
210 bioavailability was merely 11%, hence considerably lower than in the studies by Agerso  
211 and Friis (1998a, b), Anadon et al. (2000), and Martinez-Larranga et al. (2004).

212 Morthorst (2002) described the oral bioavailability of amoxicillin in weaned piglets  
213 after oral administration under different experimental conditions. A dose of 20 mg/kg  
214 amoxicillin incorporated into the daily ratio of feed or drinking water resulted in very low  
215 levels in the serum. The bioavailability ranged from 6% to 29 %. However, after a single  
216 oral dose administered in 200 ml drinking water directly into the stomach (intragastical  
217 application) in fastened pigs, Morthorst (2002) found much higher and more variable  
218 values for  $C_{\text{max}}$  ( $21.55 \mu\text{g/ml} \pm 34.55$ ), a bioavailability of 91 %, and a short  $t_{\text{max}}$  of about  
219 30 minutes (Table 3). These data indicate that the oral bioavailability of amoxicillin can  
220 be considerably reduced by interactions with feed.

221 The plasma protein binding rate of amoxicillin in pigs has been described to be  
222 28% (Villa et al., 1994). This is in agreement with the findings of Agerso and Friis

223 (1998b) which measured  $24 \pm 9\%$  in plasma and  $21 \pm 13\%$  in bronchial secretions.  
224 Protein binding measured in pigs experimentally infected with *A. pleuropneumoniae*  
225 (Agerso and Friis, 1998c) were slightly lower (plasma:  $17 \pm 8\%$ , bronchial secretions:  $13$   
226  $\pm 6\%$  in bronchial secretions). The data suggest that there is good availability of the drug  
227 in the infected tissue.

228

229

### 230 **3. Susceptibility of amoxicillin among bacteria associated with** 231 **porcine respiratory infections**

232

233 The major bacterial pathogens involved in porcine respiratory tract infections are *A.*  
234 *pleuropneumoniae*, *P. multocida*, *H. parasuis*, *B. bronchiseptica*, *S. suis*, and  
235 *Mycoplasma hyopneumoniae*. While there is currently no guideline available for the  
236 reliable performance of *in-vitro* susceptibility testing of mycoplasmas, the CLSI guideline  
237 M31-A2 (NCCLS, 2002a) describes in detail how to perform the susceptibility testing of  
238 some of the other porcine respiratory tract pathogens, e.g. *P. multocida*, *A.*  
239 *pleuropneumoniae* or *Streptococcus* spp.. Susceptibility testing of *B. bronchiseptica* and  
240 *H. parasuis* used CLSI methods as laid down in document M31-A2 with either cation-  
241 supplemented Mueller-Hinton broth (*B. bronchiseptica*) or veterinary fastidious medium  
242 (*H. parasuis*) as the test medium.

243 The studies conducted by the Federal Office of Consumer Protection and Food  
244 Safety (Bundesamt für Verbraucherschutz and Lebensmittelsicherheit, BVL) as part of  
245 the GERM-Vet national resistance monitoring program in Germany provide a reliable  
246 database on the current resistance status of selected veterinary pathogenic bacteria

247 (Wallmann et al., 2003). The GERM-Vet program follows the CLSI guidelines and  
248 determines MIC values by broth microdilution. According to the CLSI guidelines M31-A2  
249 and M31-S1, ampicillin is considered as the class representative of the  
250 aminobenzylpenicillins, of which ampicillin and amoxicillin are approved for veterinary  
251 use. As a consequence, ampicillin is commonly used to test for susceptibility to  
252 amoxicillin since the results obtained with ampicillin are also valid for amoxicillin (NCCLS  
253 2002a; 2004). Thus, in most of the studies mentioned below the isolates were tested for  
254 susceptibility against ampicillin while amoxicillin was used only in a few studies (Marie et  
255 al., 2002; Gutiérrez-Martín et al., 2006; Vela et al., 2005).

256 Among the porcine respiratory tract pathogens, susceptibility data of *P. multocida*  
257 (Wallmann et al., 2003, 2004) and *B. bronchiseptica* for ampicillin (Wallmann et al.,  
258 2004; Kadlec et al., 2004) have been published so far. The *in-vitro* susceptibility of *P.*  
259 *multocida* proved to be rather stable for isolates obtained during the years 2001,  
260 2002/2003, and 2004/2005 in Germany (Table 4). Similar MIC values have also been  
261 reported for *P. multocida* isolates from Spain collected during 1987/1988 and 2003/2004  
262 (Yanedt et al., 2006). Most of the *P. multocida* isolates exhibited low MIC values in the  
263 range of  $\leq 0.25$   $\mu\text{g/ml}$ , independently of their geographical origin. In contrast, *B.*  
264 *bronchiseptica* isolates exhibited high MIC<sub>50</sub> and MIC<sub>90</sub> values of ampicillin ranging  
265 between 8-16  $\mu\text{g/ml}$  and 16-32  $\mu\text{g/ml}$ , respectively (Table 4).

266 There are only few recently published data available for *A. pleuropneumoniae*  
267 (Matter et al., 2007; Gutiérrez-Martín et al., 2006), *S. suis* (Wisselink et al., 2006; Vela et  
268 al., 2005; Marie et al., 2002), and *H. parasuis* (Aarestrup et al., 2004; Martín de la  
269 Fuente et al., 2007). These studies, all following CLSI methodology, showed that *A.*  
270 *pleuropneumoniae* (Gutiérrez-Martín et al., 2006) and *H. parasuis* (Martín de la Fuente

271 et al., 2007) from Spain showed distinctly higher MICs for the aminopenicillins tested  
272 than isolates from Switzerland (Matter et al., 2007) and Germany or Denmark (Aarestrup  
273 et al., 2004) and UK (Martín de la Fuente et al., 2007), respectively (Table 4). In the  
274 recent GERM-Vet studies conducted by the BVL for the years 2004 and 2005 on 124 *A.*  
275 *pleuropneumoniae* isolates, the MIC<sub>50</sub> and MIC<sub>90</sub> values of ampicillin were 0.25 µg/ml.  
276 For *S. suis*, MIC<sub>50</sub> and MIC<sub>90</sub> values of ampicillin for the years 2004 and 2005 – as  
277 determined in the GERM-Vet program – were ≤ 0.03 and 0.06 µg/ml, respectively (Table  
278 4). This is also in agreement with data from Spain (Vela et al., 2005) and confirms the  
279 usual susceptibility of streptococci to penicillins. Other studies on the antimicrobial  
280 susceptibility of *S. suis* did not provide a distribution of MICs of amoxicillin (Marie et al.,  
281 2002), or tested only penicillin as the sole representative of the class of penicillins  
282 (Wisselink et al., 2006).

283

284

#### 285 **4. Clinical efficacy of amoxicillin in respiratory infections**

286

287 The efficacy of amoxicillin in diseases caused by *A. pleuropneumoniae* was  
288 confirmed in an experimental infection model and in clinical field studies. Tanigawa and  
289 Sawada (2003) investigated the pharmacodynamic effect of subinhibitory and inhibitory  
290 amoxicillin concentrations *in-vitro* and *in-vivo* using a long-acting amoxicillin formulation  
291 in pigs experimentally infected with a strain of *A. pleuropneumoniae* which exhibited an  
292 MIC of 0.39 µg/ml. The animals of group I, which were treated with a dose of 7.5 mg/kg  
293 i.m., had a slower clinical recovery than the pigs treated with a higher dose of 15 mg/kg  
294 i.m.. The comparative kinetics of the administered doses in relation to the MIC of 0.39

295  $\mu\text{g/ml}$  for the *A. pleuropneumoniae* strain suggested almost entirely subinhibitory  
296 pulmonary concentrations in group I, which explained the slower clinical recovery  
297 measured by the clinical score and the higher lung lesion score. In contrast, in group II  
298 (15 mg/kg amoxicillin i.m.) concentrations above the MIC could be expected throughout  
299 the treatment period.

300 In another experiment, Tanigawa and Sawada (2002) studied the effect of a long-  
301 acting formulation of amoxicillin in pigs. The animals were treated with amoxicillin (15  
302 mg/kg i.m.) 22 h before the experimental infection with *A. pleuropneumoniae*. In view of  
303 the pharmacokinetic data (Tanigawa and Sawada, 2002; Table 1b), the MIC (0.39  
304  $\mu\text{g/ml}$ ) was probably not reached over the whole period of time of the infection, but the  
305 authors reported that only a few animals became ill.

306 Further studies, also in *A. pleuropneumoniae*-infected pigs, were conducted by Roy  
307 et al. (2000a, 2000b). Amoxicillin (Suramox® 50) was administered orally at a dosage  
308 of 20 mg/kg/day on five consecutive days, either via feed or drinking water. The clinical  
309 efficacy of amoxicillin was demonstrated versus an untreated control group by significant  
310 reduction of clinical signs, by the absence of gross pathological changes and also by  
311 microbiological criteria. No information was provided concerning the susceptibility of the  
312 *A. pleuropneumoniae* isolates used.

313 Thomas et al. (2000) reported results from a clinical field trial with amoxicillin  
314 trihydrate (without aluminium stearate, Clamoxyl®) in comparison to marbofloxacin. The  
315 study was conducted on pig holdings in France, Germany and the Netherlands. The  
316 clinical efficacy of amoxicillin at a dose of 7 mg/kg after i.m. administration for 3-5  
317 consecutive days was 68% in comparison to 74.5% for the animals treated with  
318 marbofloxacin (7 mg/kg). No statistically significant differences were found. The typical



319 pathogens implicated in porcine respiratory disease, *A. pleuropneumoniae*, *S. suis*, *P.*  
320 *multocida*, and *M. hyopneumoniae*, were isolated, but there were no data on the  
321 susceptibility of these infectious agents to amoxicillin and no information about untreated  
322 controls.

323

324

## 325 **5. Clinical breakpoints for amoxicillin**

326

327 Most breakpoints for antimicrobial agents considered in veterinary medicine are  
328 derived from human medicine. The few internationally approved veterinary-specific  
329 clinical breakpoints established to date have been published in the CLSI documents  
330 M31-A2 (2002a) and M31-S1 (2004). No veterinary-specific breakpoints are available for  
331 ampicillin or amoxicillin in these two documents. When summarizing the data available  
332 for amoxicillin with regard to the pharmacodynamics, pharmacokinetics, clinical efficacy  
333 and *in vitro* susceptibility of the target pathogens, the following aspects must be taken  
334 into consideration.

335 From the **pharmacodynamic** point of view, amoxicillin belongs to a class of time-  
336 dependent antibiotics. This means that its concentration in the infected tissue must be  
337 above the MIC of the causative bacterial pathogen (Craig, 1998; Kietzmann et al.,  
338 2004). The time for which the antibiotic concentration must be above the MIC is  
339 determined by various factors, but it should not be less than 30-40% of the dosing  
340 interval (Craig, 1998).

341 The **pharmacokinetics** of amoxicillin in pigs have been adequately described in  
342 the literature. It can be assumed that the serum concentration is equivalent to the

343 concentration in the interstitial fluid, where most infectious agents reside (Craig, 1995).  
344 The tissue concentration of amoxicillin is therefore not necessarily the decisive  
345 parameter since the actual concentration of the antibiotic at the site of infection might be  
346 underestimated. According to studies by Agerso and Friis (1998a, b, c), the ratio of AUC  
347 tissue to AUC serum for bronchial secretion of sick animals is about 0.55. However, this  
348 does not permit the derivation of a concentration curve in bronchial secretion. Instead,  
349 the serum pharmacokinetics of the drug should be used as the basis for estimating the  
350 concentration in tissue, as suggested by Craig (1995). The predicted tissue  
351 concentration in the lungs following parenteral administration (i.v. or i.m.) of amoxicillin  
352 at a dosage of 7.5–15 mg/kg amounts to 0.5–1.0 µg/ml for about 6–12 h, depending on  
353 the formulation. This estimation is mainly based on the data described by Agerso and  
354 Friis (1998b) together with the available data on the plasma half-life time and the AUC  
355 values (Table 1b). Orally administered products have to be considered separately  
356 because of their different bioavailability and the different pharmaceutical formulations. In  
357 order to reach therapeutically relevant serum levels in plasma of 0.5–1.0 µg/ml, doses of  
358 at least 10 mg/kg twice daily are required (Agerso and Friis, 1998a, b; Agerso et al.,  
359 1998).

360 Only few data are available on the **clinical efficacy** of amoxicillin (Clamoxyl®) in  
361 pigs (Thomas et al., 2000; Tanigawa and Sawada, 2003). Thomas et al. (2000) reported  
362 the efficacy of the amoxicillin-containing product as being similar to that of marbofloxacin  
363 (Marbocyl®), but there is no information about the susceptibility of the infecting  
364 pathogens. In infection experiments done by Tanigawa and Sawada (2003), the  
365 susceptibility of *A. pleuropneumoniae* to amoxicillin, expressed as its MIC, was  
366 determined at 0.39 µg/ml. A long-acting formulation of amoxicillin produced a clinical

367 cure at dose rates of 7.5 mg/kg and 15 mg/kg i.m., but this result was achieved earlier in  
368 the group of animals given the high dose. Although it appears to be unlikely for the  
369 treatment with the lower dose, the infectious agent was not longer isolated from these  
370 animals, indicating a complete elimination of the pathogens. Therefore, it can be  
371 assumed that bacteria with an MIC of approximately 0.4 µg/ml are reliably killed and  
372 completely eliminated from the lungs. This confirms the assumption that clinical efficacy  
373 corresponds with consistent concentrations of the active ingredient in the lungs of  
374 approx. 0.5–1 µg/ml.

375 The **susceptibility** status of field isolates of porcine *P. multocida* isolated in  
376 Germany (Wallmann et al., 2003; 2004) revealed that the vast majority of the strains  
377 exhibited MICs of  $\leq 0.5$  µg/ml, suggesting that *P. multocida*-based infections in pigs can  
378 be treated reliably and efficiently with amoxicillin. The same applies – with few  
379 exceptions (Table 4) – to infections with the bacteria *A. pleuropneumoniae*, *H. parasuis*,  
380 and *S. suis*. The distribution of MIC values against *B. bronchiseptica* was also highly  
381 homogeneous with the MIC<sub>50</sub> and MIC<sub>90</sub> values of 8–16 µg/ml (Wallmann et al., 2004;  
382 Kadlec et al., 2004). However, an amoxicillin concentration which is able to inhibit the  
383 growth of *B. bronchiseptica* cannot be reached – neither in serum nor in tissue – with the  
384 approved products at the recommended dosage. Thus, amoxicillin should not be taken  
385 into consideration for therapeutic interventions against *B. bronchiseptica* infections. It is  
386 also noteworthy that *Mycoplasma hyopneumoniae* and other *Mycoplasma* spp. lack the  
387 target structure of amoxicillin, namely the cell wall. As a consequence, amoxicillin – as  
388 all other antimicrobial agents that act on the cell wall synthesis – is inactive against  
389 mycoplasmas and therefore should not be used to treat infections caused by  
390 *Mycoplasma* spp..

391 Based on all aforementioned pharmacological, clinical and microbiological  
392 parameters, we suggest the following clinical breakpoints for amoxicillin to be used for  
393 the classification of bacterial pathogens involved in porcine respiratory tract infections:  
394  $\leq 0.5 \mu\text{g/ml}$  for "susceptible",  $1.0 \mu\text{g/ml}$  for "intermediate", and  $\geq 2 \mu\text{g/ml}$  for "resistant".  
395 This recommendation takes into account the diversity and completeness of the available  
396 data with regard to the formulations, dosages and treatment intervals used. Mainly in  
397 view of the great paucity of clinical test results, the recommended breakpoint for the  
398 classification "susceptible" has been set at the lower end of the possible range between  
399  $0.5 \mu\text{g/ml}$  and  $1 \mu\text{g/ml}$ .

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401

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Table 1: Comparative description of the most important pharmacokinetic parameters in pigs after (a) intravenous (i.v.) administration and (b) intramuscular (i.m.) administration of different formulations of amoxicillin at different dose rates

(a)

<b>Intravenous administration</b>	<b>AUC (mg/h/l)</b>	<b>V<sub>ss</sub> (l/kg)</b>	<b>MRT (h)</b>	<b>Cl<sub>B</sub> (l/h/kg)</b>
Agerso and Friis (1998a) dose: 8.6 mg/kg, trial 1	23.5 ± 3.7	0.55 ± 0.05	1.5 ± 0.20	0.37 ± 0.06
Agerso and Friis (1998a) dose: 8.6 mg/kg, trial 2	17.0 ± 3.4	0.63 ± 0.17	1.2 ± 0.20	0.52 ± 0.10
Hernandez et al. (2005) dose: 15 mg/kg	4084 ± 1011 (µg/min/ml)	0.81	1.5 ± 0.42	3.9 ± 1.2 (ml/min/kg)
Martinez-Larranaga et al. (2004) dose: 20 mg/kg	67.11 ± 4.19	1.07 ± 0.08	3.54 ± 0.43	0.30 ± 0.02
Morthorst (2002) dose: 20 mg/kg	23.6 ± 2.44	n.d.*	n.d.	n.d.

\* n.d. = not determined

(b)

<b>Intramuscular administration **</b>	<b>t<sub>max</sub> (h)</b>	<b>C<sub>max</sub> (µg/ml)</b>	<b>AUC (mg/h/l)</b>	<b>MRT (h)</b>	<b>Bioavaila- bility (F)</b>
Agerso and Friis (1998a) dose: 14.7 mg/kg	2.0 ± 0.7	5.1 ± 0.8	33.1 ± 3.9	8.8 ± 2.6	0.82 ± 0.08
Morthorst (2002) dose: 20 mg/kg	1.21 ± 0.73	8.54 ± 3.4	27.8 ± 10.4	n.d.*	1.18
Tanigawa and Sawada (1998) dose: 7.5 mg/kg	n.d.	1.12 ± 0.45	21.0 ± 12.0	n.d.	n.d.
Agerso and Friis (1998a) dose: 14.1 mg/kg, LA ***	1.3 ± 0.5	1.7 ± 1.0	47.6 ± 7.0	66.8 ± 26.2	1.26 ± 0.24
Tanigawa and Sawada (1998), dose: 15 mg/kg	n.d.	2.81 ± 0.48	42.9 ± 9.93	n.d.	n.d.

\* n.d. = not determined

\*\* no values for V<sub>ss</sub> and Cl<sub>B</sub> have been determined in any of these studies

\*\*\* formulation with aluminium stearate, long-acting formulation

Table 2: Comparative description of the most important pharmacokinetic parameters in pigs after peroral (p.o.) administration (by gavage) of different formulations of amoxicillin at different dose rates

Peroral administration	$t_{\max}$ (h)	$C_{\max}$ ( $\mu\text{g/ml}$ )	AUC (mg/h/l)	$V_{ss}$ (l/kg)	MRT (h)	$Cl_B$ (l/h/kg)	Bioavailability (F)
Agerso and Friis (1998a) dose: 10.1 mg/kg * trial 1	$1.9 \pm 0.9$	$1.6 \pm 0.9$	$6.5 \pm 2.9$	n.d.	$8.7 \pm 6.2$	n.d.	$0.31 \pm 0.15$
Agerso and Friis (1998a) dose: 10.1 mg/kg trial 2	$3.6 \pm 1.5$	$0.8 \pm 0.3$	$5.5 \pm 1.5$	n.d.	$10.4 \pm 1.9$	n.d.	$0.28 \pm 0.08$
Anadon et al. (2000) dose: 20 mg/kg	$0.96 \pm 0.18$	$6.76 \pm 0.67$	$25.2 \pm 3.6$	$1.81 \pm 0.23$	n.d.	$0.3 \pm 0.03$	$0.39 \pm 0.08$
Sumano et al. (2004) dose: 20 mg/kg**	$1.66 \pm 0.02$	$3.07 \pm 0.06$	$14.4 \pm 0.16$	n.d.	n.d.	n.d.	n.d.
Sumano et al. (2004) dose: 20 mg/kg, conventional formulation A	$1.85 \pm 0.16$	$1.53 \pm 0.12$	$7.73 \pm 0.37$	n.d.	n.d.	n.d.	n.d.
Sumano et al. (2004) dose: 20 mg/kg, conventional formulation B	$1.25 \pm 0.53$	$1.67 \pm 0.24$	$7.21 \pm 0.24$	n.d.	n.d.	n.d.	n.d.

\* fasting pigs

\*\* special formulation

Table 3: Comparative description of the most important pharmacokinetic parameters in pigs after peroral (p.o.) administration (in feed or drinking water) of different formulations of amoxicillin at different dose rates

peroral administration	$t_{\max}$ (h)	$C_{\max}$ ( $\mu\text{g/ml}$ )	AUC (mg/h/l)	V <sub>ss</sub> (l/kg)	MRT (h)	Cl <sub>B</sub> (l/h/kg)	Bioavailability (F)
Anadon et al. (2000)* dose: 20 mg/kg	0.96 ± 0.18	6.76 ± 0.67	25.2 ± 3.6	1.81 ± 0.23	n.d.	0.3 ± 0.03	0.39 ± 0.08
Anfossi et al. (2002)** dose: 50 mg/kg microgranular formulation	2.5 ± 1.37	4.2 ± 2.41	18.9 ± 9.18	n.d.	4.01 ± 0.84	n.d.	n.d.
Anfossi et al. (2002)** dose: 50 mg/kg microgranular formulation	1.78 ± 0.36	3.36 ± 1.36	14.15 ± 5.43	n.d.	4.02 ± 0.75	n.d.	n.d.
Anfossi et al. (2002)** dose: 50 mg/kg	2.06 ± 1.63	2.85 ± 0.74	12.11 ± 2.4	n.d.	3.86 ± 0.81	n.d.	n.d.
Hernandez et al. (2005)** dose: 15 mg/kg	5.8 ± 2.3	0.76 ± 0.05	n.d.	n.d.		n.d.	0.11 ± 0.05
Martinez-Larranaga et al. (2004)** dose: 20 mg/kg	0.97 ± 0.29	7.37 ± 0.42	27.4 ± 4.93	1.35 ± 0.2	4.47 ± 0.30	n.d.	0.41
Morthorst (2002)*** dose: 20 mg/kg	0.55 ± 0.85	21.6 ± 34.5	21.4 ± 12.9	n.d.	n.d.	n.d.	0.98

\* oral administration not defined

\*\* in feed

\*\*\* in drinking water

Table 4: Distribution of MIC values of ampicillin or amoxicillin among relevant porcine respiratory tract pathogens

Bacteria	Time span	Country	No. of strains tested	MIC values ( $\mu\text{g/ml}$ )					MIC <sub>50</sub>	MIC <sub>90</sub>	Reference
				$\leq 0.25$	0.5	1	2	$\geq 4$			
<i>A. pleuropneumoniae</i>	1997-2004	Spain	229	28	154	10	3	34	0.5	$\geq 64$	Gutierrez-Martin et al. (2006) *
	2002-2004	Switzerland	83	66	12	2	0	3	0.25	0.5	Matter et al. (2007)
	2004-2005	Germany	124	114	4	0	0	6	0.25	0.25	Wallmann (unpublished data)
<i>H. parasuis</i>	1998-2002	Denmark	52	0	0	52	0	0	1	1	Aarestrup et al. (2004)
	1995-2005	UK	30	22	3	1	1	3	0.5	1	Martin de la Fuente et al. (2007)
	2002-2004	Spain	30	6	2	2	2	17	16	$\geq 32$	Martin de la Fuente et al. (2007)
<i>B. bronchiseptica</i>	2000-2003	Germany	349	0	0	12	6	331	8	16	Kadlec et al. (2004)
	2002-2003	Germany	138	0	0	0	3	135	16	16	Wallmann et al. (2004)
	2004-2005	Germany	241	0	0	0	1	240	16	32	Wallmann (unpublished data)
<i>P. multocida</i>	2001	Germany	176	170	1	0	1	4	0.12	0.25	Wallmann et al. (2003)
	2002-2003	Germany	442	391	13	7	6	25	$\leq 0.12$	0.5	Wallmann et al. (2004)
	2004-2005	Germany	287	275	2	1	0	9	0.12	0.25	Wallmann (unpublished data)
	1987-1988	Spain	63	62	0	0	0	1	$\leq 0.25$	$\leq 0.25$	Yanedt et al. (2006)
	2003-2004	Spain	132	119	6	1	0	6	$\leq 0.25$	$\leq 0.25$	Yanedt et al. (2006)
<i>S. suis</i>	2004-2005	Germany	312	307	1	0	2	2	$\leq 0.03$	0.06	Wallmann (unpublished data)
	1999-2001	Spain	151	145	2	2	1	1	$\leq 0.25$	$\leq 0.25$	Vela et al. (2005) *

\* In these studies, susceptibility to amoxicillin (instead of susceptibility to ampicillin) was tested.