



HAL
open science

Impact of colistin administered before or after inoculation on the transmission of a mcr-1 colistin-resistant Escherichia coli strain between pigs

Gwenaëlle Mourand, Mathieu Andraud, Eric Jouy, Claire Chauvin, Laetitia Le Devendec, Frédéric Paboeuf, Isabelle Kempf

► **To cite this version:**

Gwenaëlle Mourand, Mathieu Andraud, Eric Jouy, Claire Chauvin, Laetitia Le Devendec, et al.. Impact of colistin administered before or after inoculation on the transmission of a mcr-1 colistin-resistant Escherichia coli strain between pigs. *Veterinary Microbiology*, 2019, 230, pp.164 - 170. <10.1016/j.vetmic.2019.02.002>. <hal-03485720>

HAL Id: hal-03485720

<https://hal.science/hal-03485720v1>

Submitted on 20 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License

1

2 Impact of colistin administered before or after inoculation on the transmission of a *mcr-1*
3 colistin-resistant *Escherichia coli* strain between pigs

4

5 Gwenaëlle Mourand^{1,2}, Mathieu Andraud^{1,2}, Eric Jouy^{1,2}, Claire Chauvin^{1,2}, Laetitia Le
6 Devendec^{1,2}, Frederic Paboeuf^{1,2} and Isabelle Kempf^{1,2}

7

8 ¹ ANSES, Laboratoire de Ploufragan-Plouzané-Niort, Ploufragan, France.

9 ² Université Bretagne Loire, France

10

11 *Corresponding author

12 Isabelle Kempf

13 ANSES Laboratoire de Ploufragan-Plouzané-Niort,

14 Zoopole Les Croix

15 22440 Ploufragan

16 France

17 Telephone: 33 2 96 01 62 81

18 Fax: 33 2 96 01 62 73

19 E-mail: isabelle.kempf@anses.fr

20

21

22 Abstract

23 Colistin resistance associated with plasmidic resistance genes is a serious public health issue.

24 We aimed at studying the transmission of an *mcr-1* colistin- and rifampicin-resistant

25 *Escherichia coli* strain between inoculated pigs and sentinels in different controlled

26 conditions. Three groups of four pigs were bred in separated animal rooms and inoculated on
27 Day 0 (D0). In each inoculated group, six contact pigs were introduced on D2. The first
28 inoculated-and-contact group was left untreated. The ten pigs in the second inoculated-and-
29 contact group received colistin (100 000 IU/kg) before inoculation or contact (D-7 to D-5),
30 simulating prophylactic administration. Pigs in the third inoculated-and-contact group were
31 treated just after inoculation or before transfer (D0 to D2), simulating metaphylactic
32 administration. Faecal samples were regularly collected and segments of intestinal tracts were
33 obtained at necropsy, on D20-D22.

34 Samples were cultured on rifampicin-supplemented media, and PCR was used to detect the
35 *mcr-1* gene. The kinetics of infection, based on culture results, were analysed using an SIR
36 model.

37 The inoculated strain was detected in all inoculated and contact pigs. The SIR model showed
38 that one infected pig could transmit the resistant bacteria to one susceptible individual in less
39 than 3 hours on average. Prophylactic administration significantly enhanced the transmission
40 rate and resulted in more samples containing the *mcr-1* resistance gene at necropsy. No effect
41 of metaphylactic administration could be detected on the transmission rate, nor on the carriage
42 of the resistant strain.

43 Our study confirms that colistin should not be used in a prophylactic manner.

44

45 **Key-words**

46 Pig; colistin; resistance; *mcr-1*; transmission; treatment

47

48

49 **Introduction**

50

51 Colistin resistance in *Enterobacteriales* is a veterinary and public health concern. Since the
52 first report of the *mcr-1* plasmidic resistance gene in pigs, poultry and clinical isolates in
53 China (Liu et al., 2016), many countries have described the presence of *mcr-1* and *mcr*
54 variants in strains of human, animal, food and environmental origin (Wang et al., 2018). The
55 *mcr* genes encode a phospho-ethanolamine-transferase, leading to the alteration of the lipid A
56 subunit of the LPS, resulting in decreased attraction of polymyxins. This modification of the
57 outer membrane is associated with a rather low level of resistance. Other mechanisms of
58 resistance resulting from chromosomal mutations in the genes involved in modifications of
59 lipid A, such as *pmrA*, *pmrB*, *phoP*, *phoQ*, and *mgrB*, have also been described. Recently, we
60 showed that most colistin-resistant isolates from diseased pigs in France carry the *mcr-1* gene,
61 sometimes with chromosomal mutations (Delannoy et al., 2017).

62 Like in other cases of resistance, the prevalence of colistin resistance in animals is probably
63 associated with the use of polymyxins, or other antimicrobials in the case of co-selection of
64 multi-drug-resistant strains (Haenni et al., 2016). The resistance gene, being carried by
65 plasmids, can be transmitted both vertically through cell division or through conjugations
66 between bacteria in animate and inanimate environments (Volkova et al., 2013). Between-
67 host transmission of resistant bacteria therefore occurs by ingestion of contaminated faeces
68 released into the environment by colonised animals. Selection pressure exerted by
69 antimicrobial administration may also affect the transmissibility of resistant bacteria between
70 animals. Therefore, a specific experimental trial was developed to quantify the transmission
71 of an *mcr-1*-positive *E. coli* strain from inoculated to sentinel pigs. To evaluate the impact of
72 colistin administration and to better cover diverse field conditions, different experimental

73 designs were implemented, with colistin given or not given to the inoculated or exposed
74 animals before or just after inoculation of the resistant strain.

75

76 **Material and methods**

77 **Animals and animal facilities**

78 The trial was performed in accordance with French animal welfare regulations and the
79 protocol approved by the ANSES/ENVA/UPEC Ethics Committee (ComEth authorisation 16-
80 053 (APAFIS No. 2016060108594266). The trial was conducted at the ANSES Ploufragan
81 animal facilities. Strict biosecurity measures were implemented in order to avoid
82 contamination of the pigs, including the use of an air filtration system and airlocks for each
83 unit, unit-specific clothes, and compulsory showering after visiting the pigs. The trials were
84 conducted with specific-pathogen-free (SPF) Large White piglets.

85 The eight-week-old piglets were randomised before the experiment. The animals did not
86 receive any antibiotic treatment prior to the trial and were given the same non-supplemented
87 feed. Each room contained two pens.

88 The names of the groups are encoded according to their status (T for treated, NT for non-
89 treated, I for inoculated, and E for exposed) and the time (in days) of the events (for example,
90 T-7E2 means animals treated from Day -7 and exposed to inoculated animals on Day 2).

91 The experimental design (Figures 1 and 2) was built to simulate transmission of a resistant
92 strain from contaminated (inoculated) non-treated animals (NTI0), from animals treated and
93 then inoculated (treated-inoculated (T-7I0)), or from animals inoculated and then treated
94 (T0I0) to groups of contact pigs. The respective contact groups were NTE2 (non-treated pigs,
95 put in contact with NTI0 on Day 2), T-7E2 (pigs treated from Day -7 and then introduced on
96 Day 2 into the room of the T-7I0 group), and T0E2 (pigs treated from Day 0 and then
97 introduced on Day 2 into the room of the T0I0 group).

98 The NTI0 / NTE2 setting simulates the transmission of a resistant strain in non-treated
99 animals.

100 The T-7I0 / T-7E2 setting parallels prophylactic administration, when the drug is given to a
101 group of animals before they are exposed to a pathogen. This situation was used to evaluate
102 the colonisation by the resistant strain of inoculated pigs whose microbiota had previously
103 been altered by colistin and might still contain low colistin concentrations, and the subsequent
104 transmission of this strain to the microbiota of previously treated exposed pigs (T-7E2).

105 The T0I0 / T0E2 setting corresponds to metaphylactic administration, when the drug is given
106 to animals, some of which are already contaminated (T0I0), while the others may be exposed
107 in the following days (T0E2). In this case, the resistant strain is inoculated to pigs when the
108 colistin concentration in the microbiota is high and alters the colistin-susceptible
109 *Enterobacteriales* population (Fleury et al., 2016) and, depending on the colistin
110 concentration in the gut, might also inhibit the colistin-non-susceptible *Enterobacteriales*
111 population.

112 At the beginning of the trial (Days -27 to Day 2), the pigs were dispatched in six animal
113 rooms, as indicated in Figure 1. Pigs in groups T-7NI, T-7I0, and T-7E2 received colistin
114 (Colivet solution, 2 000 000 IU/mL, CEVA, Libourne, France) by oral gavage at the
115 recommended dosage (50 000 IU/kg of body weight twice a day) from Day -7 to Day -5. On
116 Day 0, pigs in groups NTNI and T-7NI were orally inoculated with 10 mL of sterile broth. On
117 the same day, pigs in groups NTI0, T-7I0 and T0I0 were orally inoculated with 10 mL of the
118 *mcr-1* positive UB10-260Rif *E. coli* inoculum (Mourand et al., 2018). Pigs in groups T0E2 and
119 T0I0 were given colistin on Days 0, 1 and 2, as previously described. On Day 0, the colistin
120 was given at 10 AM and 5 PM, whereas the inoculation was performed at 11 AM. On Day 2,
121 the exposed pigs were moved, as indicated in Figures 1 and 2. Thus pigs NTE2, T-7E2 and
122 T0E2 were introduced into rooms R4 to R6, respectively.

123 During the week, daily clinical examinations consisted of looking for general clinical signs
124 and taking rectal temperatures. Individual faecal samples were collected from all pigs before
125 the beginning of the trial, and on Days 4 to 8, 10, 13, 17 and 20. Inoculated pigs were also
126 sampled on Days 1 and 2 at 12 AM, whereas exposed pigs were sampled on Day 2, four hours
127 after their introduction into rooms R4 to R6.

128 The pigs were euthanised on Days 20 (pigs NTNI, T-7NI, T0I0 and T0E2), 21 (pigs T-7I0 and
129 T-7E2) and 22 (pigs NTI0 and NTE2) and necropsied. Faecal samples obtained at different
130 levels of the digestive tract (jejunum, ileum, caecum and colon) were collected.

131

132 **Bacterial strain and media**

133 As in a previous trial (Mourand et al., 2018), we used a rifampicin-resistant mutant (UB10-
134 260Rif) of a previously characterized *E. coli* strain UB10-260, which was isolated from a
135 diseased pig in 2010 (Delannoy et al., 2017). Briefly, the strain belongs to phylogenetic group
136 A and is resistant to colistin (minimum inhibitory concentration (MIC): 4 mg/L), rifampicin,
137 sulphamethoxazole, trimethoprim, tetracycline, chloramphenicol and ampicillin. It contains
138 the *mcr-1* gene, and two mutations in the PmrB protein sequence (S138N and V351I), whose
139 impact on colistin resistance is unknown (Delannoy et al., 2017). No virulence factors could
140 be detected. *In vitro*, it has been shown to be conjugative and able to transfer the *mcr-1* gene
141 along with resistance to tetracycline, chloramphenicol and ampicillin (Delannoy et al., 2017).
142 As previously described, the inoculum was prepared by culture in Mueller Hinton (MH) broth
143 containing colistin (2 mg/L) and the bacterial pellet obtained after centrifugation was re-
144 suspended in peptone buffered solution, so as to obtain for each pig, a 10 mL inoculum
145 containing 9×10^8 CFU.

146 **Samples analysis**

147 Bacteriological analysis: faecal samples obtained during the trial or at post-mortem (PM)
148 were diluted 1/10 in peptone buffered solution containing 20% glycerol. The diluted aliquots
149 were stored at -70°C. Before freezing, 100 µL of the decimal dilutions prepared from the
150 faecal suspensions (10^{-1} to 10^{-3}) were deposited using EasySpiral® Dilute (Interscience, Saint-
151 Nom-la-Bretèche, France) onto MH plates containing 250 mg/L rifampicin (MHR). The
152 plates were incubated at 37°C for 24 h, and colonies were enumerated using the Scan 1200®
153 apparatus (Interscience). For negative samples, 200 µL of the 1/10 thawed dilutions was
154 enriched overnight in 1.8 mL of colistin-supplemented broth (2 mg/L) before plating 10 µL
155 onto MHR plates. The limit of detection was 10 CFU/g. To check that the colonies growing
156 on MHR belonged to the inoculated strain, one random colony from each pig sampled on
157 Days 1, 2, 4 and 17 was analysed: cellular lysates were prepared (Kellogg et al., 1990) and
158 PCR tests were used to confirm that isolates belonged to the *E. coli* species (Furet et al.,
159 2009), and to the same phylogenetic group as UB10-260Rif *E. coli* (Clermont et al., 2013),
160 and the presence of the *mcr-1* gene was screened for by PCR (Liu et al., 2016).
161 The *mcr-1* gene was also screened for by real-time PCR in DNA prepared from all faecal
162 samples, as previously described (Mourand et al., 2018). For all PM samples, enrichment was
163 prepared and the *mcr-1* gene was tested for by PCR. Briefly, PM samples were diluted 1/10 in
164 peptone buffer and 200 µL were added to 1.8 mL of LB broth containing 2 mg/L colistin.
165 After incubation for 24 h at 37°C, the suspensions were centrifuged for 10 min at 5 180 g and
166 a cellular lysate was prepared (Kellogg et al., 1990) and submitted to *mcr-1* qPCR (Mourand
167 et al., 2018).

168 **Estimation of transmission parameters**

169 The kinetics of infections observed in each group were analysed using an SIR model (Gallien
170 et al., in press). At each sampling time, the individuals were classified according to their
171 bacteriological results (after enrichment) as susceptible (negative), infectious (shedding

172 resistant bacteria), or recovered after the last positive sample. The actual infection times T_{Inf} ,
 173 since they were not observed, were drawn from uniform distribution ranging between the
 174 times of the last negative and the first positive samples. The probability of infection π on a
 175 time interval Δt is expressed in terms of prevalence (proportion of shedding animals) as

$$176 \quad \pi = 1 - \exp\left(-\frac{\beta I}{N} \Delta t\right),$$

177 where β is the transmission rate (average number of new infections produced by one
 178 infectious individual per unit of time). With these notations, the probability of observing the
 179 infection kinetics given the parameters β and T_{Inf} , also called likelihood function, is
 180 expressed as:

$$181 \quad L(\mathbf{T}_{obs}, I, N | \beta, \mathbf{T}_{Inf})$$

$$182 \quad = \prod_{i=1}^{N_c} \prod_{T_j=T_{obs_1}}^{T_j=T_{LastNeg_i}} \exp\left(-\frac{\beta I_j}{N} (T_j - T_{j-1})\right)$$

$$183 \quad * \left\{ 1 - \exp\left(-\frac{\beta I_{Inf}^{(i)}}{N} (T_{Inf}^{(i)} - T_{lastNeg_i})\right) \right\},$$

184 where I_j corresponds to the number of shedding animals over time interval $[T_{obs_{j-1}}, T_{obs_j}]$,
 185 and $I_{Inf}^{(i)}$ the number of shedders over the time interval during which individual i was infected.
 186 Parameter inference was performed using the Metropolis algorithm with uniform proposal
 187 distribution for the infection times and log-normal distribution for the transmission parameter.
 188 Non-informative priors were used with uniform and log-uniform distributions on large
 189 intervals ($[-4,4]$ for $\log(\beta)$ and $[T_{LastNeg}, T_{FirstPos}]$ for T_{Inf}). Three Markov chains were run
 190 with 10 000 iterations and a 1 000-iteration burn-in period, for which initial values were

191 randomly drawn from prior distributions. Algorithm convergence was assessed using classical
192 tests (Gelman-Rubin, Heidelberger, Geweke).

193

194 **Statistical analysis**

195 The Kruskal–Wallis non-parametric test was used to compare the weight gain of animals
196 present in rooms R3 to R6 at Day 20. The distributions of pigs or isolates were analysed using
197 the Chi2-test or the Fisher exact test when $n \leq 5$ (<https://biostatgv>).

198 The culture titres were Log10 transformed, and the titres of samples positive only after
199 enrichment were set at 1; titres of samples negative after enrichment were set at 0.

200

201 **Results**

202 **Clinical signs and growth**

203 No clinical signs were observed, except in one pig from the T0I0 group that suffered an
204 accident and was euthanised on Day 10. The weight gains from Day -7 to Days 2, 7, 16 and
205 20 were compared between pigs in rooms R3 to R6. No statistically significant difference was
206 detected ($p > 0.05$).

207 **Culture results: fecal samples**

208 Faecal samples collected from control pigs or collected before inoculation or transfer of
209 contact pigs were negative. Titres obtained on MHR media for samples obtained from
210 inoculated and contact pigs are given in Table 1. Analysis of 86 isolates obtained from live
211 pigs directly or after enrichment showed that all were *E. coli* and that they shared the
212 characteristics of UB10-260Rif *E. coli*: resistance to rifampicin, presence of the *mcr-1* gene,
213 and the same phylogenetic group.

214 All inoculated pigs excreted the UB10-260Rif *E. coli* strain on all dates, except one pig in the
215 T-7I0 group on Day 13, and one pig in the T0I0 group on Day 17 (Table 1). Thus, no

216 significant differences between the numbers of positive samples were found between
217 inoculated animals, either non-treated or treated before or after inoculation. Concerning
218 exposed pigs, all were positive on at least one occasion. The numbers of positive samples for
219 the NTE2, T-7E2 and T0E2 groups were 53/66 (80%), 38/66 (58%) and 49/66 (74%) (Chi2,
220 $p=0.01$), respectively. This shows that significantly fewer samples from the pigs treated
221 before their transfer (T-7E2 group) contained the inoculated strain compared to the other
222 exposed animals, being either non-treated or treated from Day 0.

223 The daily titres in the three inoculated groups (NTI0, T-7I0 and T0I0) did not differ
224 significantly, except on Day 4, when pigs in group T-7I0 excreted significantly less resistant
225 bacteria (Table 2). In each group, the maximum daily means (6.45, 5.93 and 5.82 Log₁₀/g of
226 faeces) were observed on the first day after inoculation, and decreased thereafter, but most
227 often remained higher than 3 Log₁₀/g up to the last sampling Day (Day 20). Conversely, for
228 contact pigs, means of daily titres never reached 3 Log₁₀/g, the maximum means being 2.8 on
229 Day 8, 2.2 on Day 3, and 2.8 on Day 6 for groups NTE2, T-7E2 and T0E2, respectively. A
230 comparison of these sentinel groups shows that the titres were significantly different on Days
231 4, 5 and 8, with lower titres in group T-7E2. On these days, the mean titres in this group were
232 at least 1.2 to 1.8 Log₁₀ lower than the mean titers in the other exposed groups.

233 **Culture results: PM samples**

234 Organs from all non-inoculated pigs were negative on MHR. For organs from inoculated and
235 exposed pigs (Table 3), the ratio of positive jejunum samples was significantly lower than in
236 other organs ($p<0.001$), with only three samples from T0I0 or T0E2 animals being positive.

237 No significant differences were seen between the other types of samples.

238 No significant difference in the ratio of total positive samples in the different groups of
239 inoculated pigs could be detected ($p>0.05$). This was also true for the groups of exposed pigs.

240 Overall, 34 out of 48 samples (71%) from inoculated animals were positive, thus significantly
241 more than for the exposed pigs (30 out of 72 (42%), $p=0.002$). Comparison of the titres
242 obtained for each organ according to the different inoculated groups on the one hand, and the
243 different exposed groups on the other, did not reveal any significant differences (data not
244 shown).

245 ***mcr-1* PCR on DNA prepared from faecal samples**

246 All the samples tested positive for the PCR internal control. All samples collected from non-
247 inoculated pigs or before inoculation or exposure were negative for the *mcr-1* gene. A total of
248 19/339 samples were found to be positive (Table 1), all from inoculated pigs, and mostly
249 during the first days after inoculation (9 on Day 1, 5 on Day 2, 3 on Day 3, 1 on Day 4, and 1
250 on Day 17). They were overall equally distributed between the groups, with 7 positive in the
251 NT10 group, and 6 in the T-7I0 and T0I0 groups. All *mcr-1*-positive samples were also
252 positive according to culture on MHR, except for one faecal sample from pig 6392 (group T-
253 7I0) on Day 17.

254 ***mcr-1* PCR on enrichment broths from PM samples**

255 A total of 58/120 enrichment broths prepared from PM samples were found to be positive by
256 PCR (Table 4). Jejuni were rarely positive, whereas 50% or more of the other organs were
257 positive. No significant differences were found between the total numbers of positive samples
258 in the inoculated groups. For the contact groups, the T-7E2 group yielded significantly more
259 positive PCR results (16/24, 67%) than the other two exposed groups (NTE2: 9/24 (37.5%)
260 and TOE2: 7/24 (29%)) ($p=0.02$). In all, 26 out of 48 samples (54%) from inoculated pigs
261 were found to be positive compared to 32 out of 72 (44%) for exposed animals ($p>0.05$). For
262 the inoculated and exposed groups, the numbers of positive samples detected by culture and
263 by PCR did not differ significantly.

264 **Transmission**

265 The transmission rate from the kinetics of infection observed in group T-7E2 was estimated to
266 be 0.36 [0.12; 0.88](animal/hour) (Figure 3). In other words, one infected individual would
267 be able to transmit resistant bacteria to one susceptible individual in less than 3 hours on
268 average. The estimates obtained in the other groups were significantly lower ($\beta =$
269 0.08 [0.03; 0.17] and $\beta = 0.06$ [0.02, 0.12] in groups NTE2 and T0E2, $p < 0.05$). All animals
270 in group T-7E2 were infected within one day post-contact, leading to large credibility
271 intervals for the parameter estimate, although convergence of the algorithm was achieved.
272 The average time between infection and detection ($T_{firstPos} - T_{Inf}$) ranged between 5 and 7
273 hours, with 50% of the animals being infected within 3 hours before detection, reflecting the
274 proportion of positive animals at the first sampling time (4 hours post-contact). However, the
275 results demonstrated significantly earlier infections in the pre-treated group (T-7E2; Cox
276 proportional hazard model: HR=0.73 [0.68, 0.78]), even though the exposure duration was
277 similar in all groups.

278

279 **Discussion**

280 As previously observed (Mourand et al., 2018), inoculation with UB10-260Rif *E. coli* did not
281 lead to clinical signs. After the inoculation of 9×10^8 CFU to the eight-week old pigs, the strain
282 was regularly excreted by most inoculated pigs during the three-week trial, as observed in our
283 previous assay using an inoculum of 2.5×10^8 CFU for seven-week-old pigs (Mourand et al.,
284 2018).

285 No attempt to analyse faecal or PM samples with colistin-supplemented media was performed
286 because of the poor sensitivity of such media based on our experience in previous trials
287 (Mourand et al., 2018). Similarly, *mcr-I* qPCR on DNA extracted from faecal samples, which
288 could be a measure of all *mcr-I*-containing bacteria, showed very poor sensitivity compared
289 to culture on MHR media, with only 19 positive results out of 339 (5.6%) tested samples,

290 compared to 279/339 (82.3%) for culture ($p=5 \times 10^{-90}$). Conversely for PM samples, results
291 from cultures on MHR media (53.3% positive samples) and PCR on enrichment broths
292 (48.3% positive samples) were not significantly different, probably because the PCR was
293 performed on cultures obtained after enrichment in colistin broths, resulting in a higher
294 numbers of *mcr-I*-containing cells. Thus to determine transmission parameters from fecal
295 samples, we favoured results obtained from culture on MHR media, although this medium did
296 not enable us to detect possible transfers of the *mcr-I* gene from the inoculated strain to other
297 commensal *Enterobacteriales*, as could be previously observed *in vitro* (Delannoy et al.,
298 2017) or *in vivo* (Mourand et al., 2018). This means that the calculated transmission
299 parameters are relevant for the inoculated strain and may underestimate transmission of
300 colistin-resistant strains as we took into account only the inoculated strain, and not all the
301 bacteria that may contain the *mcr-I* gene. Inversely, the loss of the *mcr-I* gene from UB10-
302 260Rif *E. coli* is probably a rare event *in vivo* as it was not detected during the
303 characterisation of all the 86 rifampicin-resistant *E. coli* re-isolated from this study, or the 261
304 samples obtained during the previous assay (Mourand et al., 2018). This means that the
305 calculated transmission parameters probably do not overestimate the transmission of colistin-
306 resistance.

307 Inoculated pigs had high titers compared to exposed pigs. This may be related to the titer of
308 the inoculated culture (9×10^8 CFU per pig), probably much higher than the doses to which
309 contact pigs were exposed, via contaminated feces from inoculated animals.

310 The comparison of faecal, PM cultural, and molecular results obtained for the different
311 inoculated groups showed that the only significant difference was observed on Day 4, with T-
312 7I0 pigs (treated on Days -7, -6 and -5) excreting less resistant bacteria compared to non-
313 treated pigs or pigs treated on Days 0, 1 and 2. We could not determine the concentration of
314 colistin in the gut of T-7I0 pigs at the time of inoculation, but Viel et al. (Viel et al., 2017)

315 reported mean concentrations of $1.8 \pm 0.3 \mu\text{g/g}$ of feces collected on Day 7 for pigs receiving
316 the recommended dose (100 000 IU/kg/day) from Day 0 to Day 4. Thus the colistin
317 concentrations in our pig guts five days after the last administration were probably very low.
318 Thus we can hypothesise that the lower excretion of the inoculated strain could be due to an
319 indirect effect of colistin, possibly associated with microbiota disruption and a more
320 heterogeneous microbial population in treated animals as observed by Fleury et al. (Fleury et
321 al., 2016). However no attempt to characterize the composition of the pig gut microbiota was
322 done.

323 Similarly for sentinel pigs, group T-7E2 differed significantly from the other exposed groups,
324 with a lower number of culture-positive faecal samples, and lower titres on Days 4, 5 and 8.
325 However, the transmission parameter was significantly higher in this group of pigs treated
326 seven days before inoculation and 9 days before exposure, suggesting higher receptivity of
327 this group of exposed pigs, again probably in relation with microbiota disruptions induced by
328 the preceding treatment. Moreover, at PM on Day 21, according to PCR on enrichment
329 broths, this group yielded more positive samples than the other exposed groups. The
330 discrepancy between the PM non-significantly-different culture results and the significantly-
331 different PCR results, obtained on enrichment from organ samples, may lie in the sampling
332 time, sampling method (faecal material or internal organs) and also in the target (only the
333 inoculated strain for culture), but all *mcr-1*-positive bacteria for the PCR. Thus, as previously
334 explained, we can hypothesise that at Day 2, i.e. 7 days after the last administration, the
335 concentration of colistin was very low (Viel et al., 2017), and that the disruption of the
336 intestinal microbiota of pigs treated on Days -7 to -5 resulted in lower colonisation during the
337 very first few days after contact on Day 2; but ultimately, at PM, more *mcr-1*-resistant
338 bacteria were present in the pigs that had received early administration of colistin. As a
339 consequence of our findings, we can imagine that under field conditions, the higher

340 contamination of internal organs by *mcr-1*-positive strains might result in more frequent
341 contaminations of meat products by resistant isolates. Moreover, the higher transmission rate
342 in case of early treatment may result in more pigs becoming contaminated, and thus in a
343 higher impact on environmental contamination. Therefore, our observations reinforce the
344 recommendations of the EMA/CVMP/ 231573/2016 not to use colistin in a prophylactic
345 manner

346 ([http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/07/WC](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/07/WC500211080.pdf)
347 [500211080.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/07/WC500211080.pdf)).

348 The absence of differences between NT10 and T010 pigs means that the colistin treatment,
349 given at the therapeutic dose during the first days of infection, had no detectable impact on the
350 colonisation of the pigs by colistin-resistant UB10-260Rif *E. coli*. The colistin MIC for
351 UB10-260Rif *E. coli* is 4 mg/L which is probably too high to inactivate the bacterium at the
352 therapeutic dosage of 50 000 IU/kg BW twice a day. In *in vitro* pharmacodynamic studies,
353 Guyonnet et al. (Guyonnet et al., 2010) showed that absence of re-growth of *E. coli* strains
354 between 5 and 24 h required concentrations of 16 to 32 times the MIC of the strain, meaning
355 that 64 to 128 mg/L are probably needed to completely inhibit the *in vitro* growth of UB10-
356 260Rif *E. coli*. They also reported that after administration of 50 000 IU/kg, the gastro-
357 intestinal tract concentration peaked at 43.6 mg/kg and a mean of 37.2 mg/kg at 0.5 h was
358 achieved for a few hours. Viel et al. (Viel et al., 2017) found colistin mean concentration
359 values of 93 to 262 mg/kg of faeces during the 5 days of administration of 100 000 IU/kg/day
360 to pigs; however, no selective effect on the inoculated *mcr-1*-positive strain, presenting a MIC
361 of 8 mg/L, was observed in their experiment, which they attributed to the fact that the bacteria
362 were exposed for too short a period to concentrations within the selection window. Similar
363 phenomena probably explain why, in our conditions, the therapeutic administration of colistin
364 did not prevent colonisation by *mcr-1*-positive UB10-260Rif *E. coli*, but inversely did not

365 induce its selection. As the colistin MICs of most *E. coli* isolates harboring an *mcr-1* gene are
366 slightly above the colistin cutoff (2 mg/L), it is probable that colistin given at the therapeutic
367 dose does not select these resistant isolates but cannot have a protective effect against an
368 intestinal infection caused by such isolates. Our observations, in line with Viel et al. (Viel et
369 al., 2017), can be compared to the results of the *in vivo* experiment reported by Liu et al. (Liu
370 et al., 2016) that used a mice thigh model. In this mice model, MCR-1 was shown to provide
371 protection against colistin, but intravenous administration of colistin was still able to decrease
372 the resistant population by more than one Log. However, this mice model differs in many
373 ways (site of infection, route of administration, presence of microbiota, etc.) from our
374 conditions.

375

376 **Conclusion**

377 The design of the trial allowed us to evaluate the transmission parameters and the persistence
378 of *mcr-1*-positive commensal *E. coli* in pigs, either inoculated with or exposed to this strain
379 and given colistin or not. The administration of colistin simulated either prophylactic or
380 therapeutic administration. Our results showed that the prophylactic administration enhanced
381 the transmission rate of the inoculated strain, and resulted at PM in more samples containing
382 the *mcr-1* resistance gene. In our conditions, no effect of therapeutic administration could be
383 detected on the transmission rate, nor on the persistence of resistance. However, other
384 experiments using different strains and experimental models are needed to further evaluate the
385 efficacy and selection impact of colistin administration in field conditions, in particular when
386 administered in cases of post-weaning diarrhoea.

387

388 **Conflict of interest statement:** the authors indicate that there are no conflicts of interest

389 **Acknowledgments**

390 The authors would like to sincerely thank Yann Bailly (ANSES Ploufragan) for technical
391 assistance.

392 **Funding:** this research was supported by the Ecoantibio Plan—a public policy set up by the
393 French Ministry of Agriculture, Agro-Food and Forestry to reduce the risks of antibiotic
394 resistance in veterinary medicine— ANSES (the French Agency for Food, Environmental and
395 Occupational Health & Safety) and the Côtes d’Armor General Council.

396 The funders had no role in study design, data collection and interpretation, or the decision to
397 submit the work for publication.

398

399

References

- Clermont, O., Christenson, J.K., Denamur, E., Gordon, D.M., 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environment. Microbiol. Reports* 5, 58-65.
- Delannoy, S., Le Devendec, L., Jouy, E., Fach, P., Drider, D., Kempf, I., 2017. Characterization of Colistin-Resistant *Escherichia coli* Isolated from Diseased Pigs in France. *Front. Microbiol.* 8, 2278.
- Fleury, M.A., Jouy, E., Eono, F., Cariolet, R., Couet, W., Gobin, P., Le Goff, O., Blanquet-Diot, S., Alric, M., Kempf, I., 2016. Impact of two different colistin dosing strategies on healthy piglet fecal microbiota. *Res. Vet. Sci.* 107, 152-160.
- Furet, J.P., Firmesse, O., Gourmelon, M., Bridonneau, C., Tap, J., Mondot, S., Dore, J., Corthier, G., 2009. Comparative assessment of human and farm animal faecal microbiota using real-time quantitative PCR. *FEMS Microbiol. Ecol.* 68, 351-362.
- Gallien, S., Andraud, M., Moro, A., Lediguerher, G., Morin, N., Gauger, P.C., Bigault, L., Paboeuf, F., Berri, M., Rose, N., Grasland, B., 2018. Better horizontal transmission of a US non-InDel strain compared with a French InDel strain of porcine epidemic diarrhoea virus. *Transbound. Emerg. Dis.* 65, 1720-1732.
- Guyonnet, J., Manco, B., Baduel, L., Kaltsatos, V., Aliabadi, M.H., Lees, P., 2010. Determination of a dosage regimen of colistin by pharmacokinetic/pharmacodynamic integration and modeling for treatment of G.I.T. disease in pigs. *Res. Vet. Sci.* 88, 307-314.
- Haenni, M., Métayer, V., Gay, E., Madec, J.Y., 2016. Increasing trends in *mcr-1* prevalence among extended-spectrum- β -lactamase-producing *Escherichia coli* isolates from French calves despite decreasing exposure to colistin. *Antimicrob. Agents Chemother.* 60, 6433-6434.
- Kellogg, D.E., Sninsky, J.J., Kwok, S., 1990. Quantitation of HIV-1 proviral DNA relative to cellular DNA by the polymerase chain reaction. *Anal. Biochem.* 189, 202-208.

- Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L.F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.H., Shen, J., 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* 16, 161-168.
- Mourand, G., Jouy, E., Chauvin, C., Le Devendec, L., Paboeuf, F., Kempf, I., 2018. Dissemination of the mcr-1 colistin resistance gene among pigs: An experimental study. *Vet. Microbiol.* 221, 122-128.
- Viel, A., Henri, J., Perrin-Guyomard, A., Laroche, J., Couet, W., Gregoire, N., Laurentie, M., 2017. Lack of experimental evidence to support mcr-1-positive escherichia coli strain selection during oral administration of colistin at recommended and higher dose given by gavage in weaned piglets. *Int. J. Antimicrob. Agents.* 51, 128-131.
- Volkova, V.V., Lu, Z., Lanzas, C., Grohn, Y.T., 2013. Evaluating targets for control of plasmid-mediated antimicrobial resistance in enteric commensals of beef cattle: a modelling approach. *Epidemiol. Infect.* 141, 2294-2312.
- Wang, R., van Dorp, L., Shaw, L.P., Bradley, P., Wang, Q., Wang, X., Jin, L., Zhang, Q., Liu, Y., Rieux, A., Dorai-Schneiders, T., Weinert, L.A., Iqbal, Z., Didelot, X., Wang, H., Balloux, F., 2018. The global distribution and spread of the mobilized colistin resistance gene mcr-1. *Nat. Commun.* 9, 1179.

Figure 1: Experimental design before and after transfer of exposed pigs on Day 2

A: Experimental design before transfer

Room	Pen 1	Pen 2
R1	6 non-treated, future-exposed pigs (NTE2)	
R2	6 treated, future-exposed pigs (T-7E2) Treatment on Days -7, -6 and -5	6 treated, future-exposed pigs (T0E2) Treatment on Days 0, 1 and 2
R3	2 control pigs (NTNI)	2 treated, non-inoculated pigs (T-7NI)

B: Experimental design after transfer of exposed pigs on Day 2

Room	Pen 1	Pen 2
R3	2 control pigs (NTNI)	2 treated, non-inoculated pigs (T-7NI)

R4	2 non-treated, inoculated pigs (NTI0)	2 non-treated, inoculated pigs (NTI0)
R5	2 treated, then inoculated pigs (T-7I0)	2 treated, then inoculated pigs (T-7I0)
R6	2 inoculated, then treated pigs (T0I0) Treatment on Days 0, 1 and 2	2 inoculated, then treated pigs (T0I0) Treatment on Days 0, 1 and 2

R4	2 non-treated, inoculated pigs (NTI0) 3 non-treated, exposed pigs (NTE ₂)	2 non-treated, inoculated pigs (NTI0) 3 non-treated, exposed pigs (NTE ₂)
R5	2 treated, then inoculated pigs (T-7I0) 3 treated, exposed pigs (T-7E2)	2 treated, then inoculated pigs (T-7I0) 3 treated, exposed pigs (T-7E2)
R6	2 inoculated, then treated pigs (T0I0) 3 treated, exposed pigs (T0E2) Treatment on Days 0, 1 and 2	2 inoculated, then treated pigs (T0I0) 3 treated, exposed pigs (T0E2) Treatment on Days 0, 1 and 2

Figure 2: Chronogram of the experiment

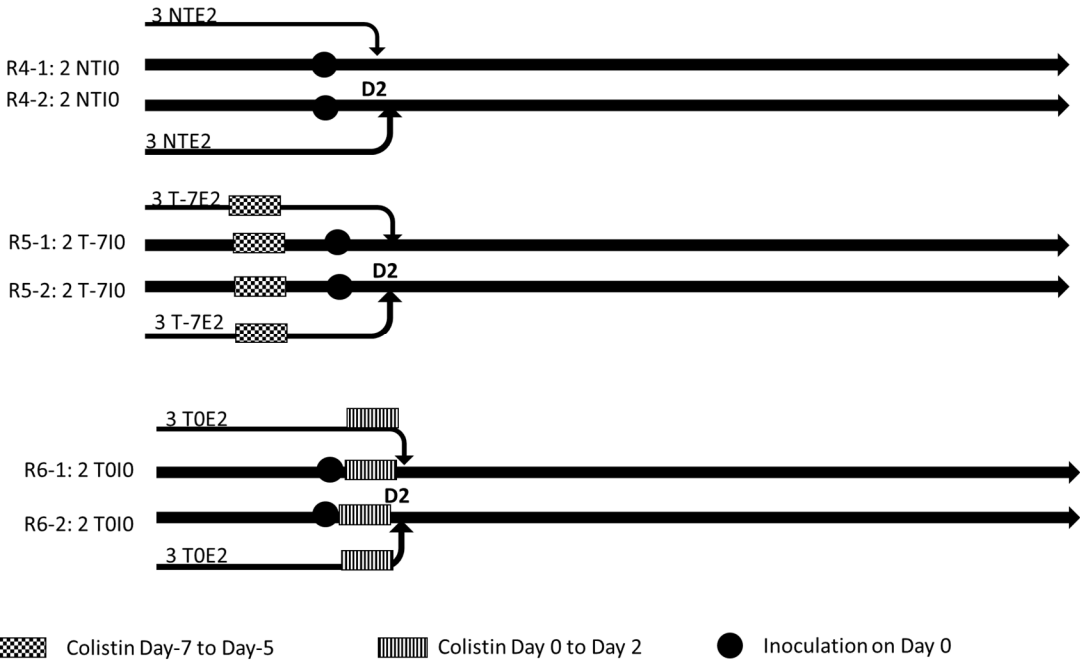


Figure 3: Distribution of transmission rates according to treatment.

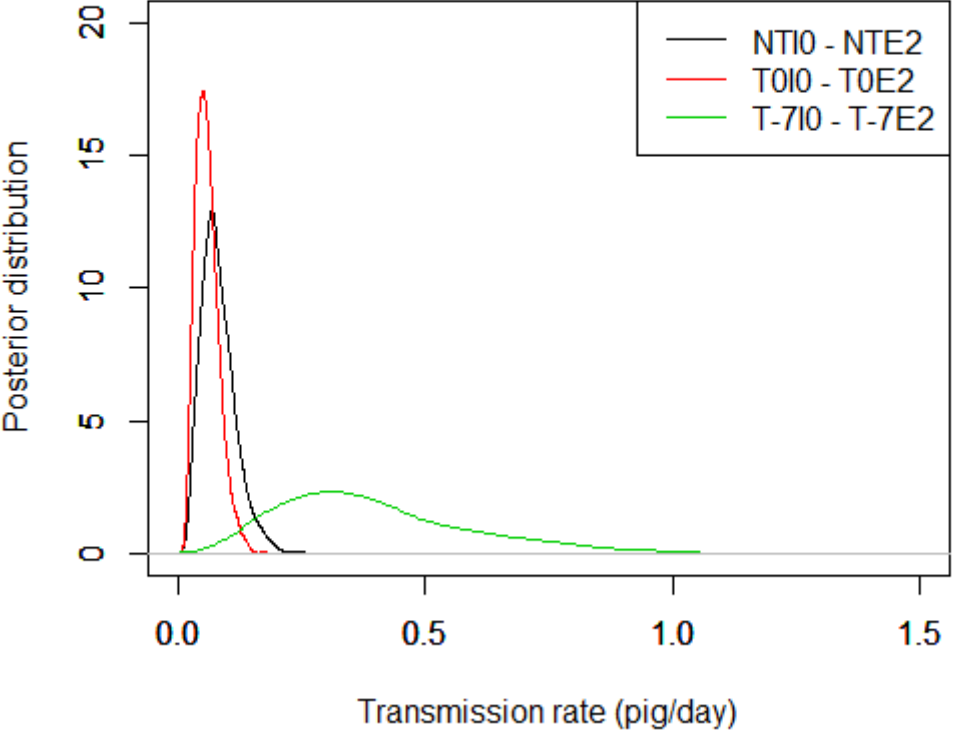


Table 1: Culture on rifampicin-supplemented media of faecal and post-mortem samples, and *mcr-1* PCR results

Room	Pen	Pig	Group	D1	D2 H0	D2 H4	D3	D4	D5	D6	D7	D8	D10	D13	D17	D20	Jejunum	Ileum	Caecum	Colon	
R4	1	6390	NTI0	P*	P	X	P	P	P	P	P	P	P	P	P	P	N	P*	P	P*	
		6450	NTI0	P*	P*	X	P*	P	P	P	P	P	P	P	P	P	P	N	P*	P*	P*
		6411	NTE2	X	X	P	P	P	P	P	P	P	P	P	P	P	P	N	P*	P*	P*
		6445	NTE2	X	X	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P*	P*
		6462	NTE2	X	X	N	P	P	P	P	P	P	P	P	P	P	PE	N	N	P*	P*
R4	2	6418	NTI0	P*	P*	X	P	P	P	P	P	P	P	P	P	P	N	P*	P	P*	
		6465	NTI0	P*	P	X	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P*
		6437	NTE2	X	X	N	P	P	PE	N	P	P	P	N	PE	P	N	N	N	N	P*
		6449	NTE2	X	X	P	PE	P	PE	N	N	P	N	P	P	P	N	N	N	N	N
		6455	NTE2	X	X	N	N	N	PE	N	PE	P	PE	P	N	PE	N	N	N	N	P*
R5	1	6392	T-7I0	P*	P	X	P	P	P	P	P	P	PE	N	N*	P	N	*	*	P*	
		6413	T-7I0	P	P*	X	P	P	P	P	P	P	P	P	P	P	N	P*	P*	P*	
		6422	T-7E2	X	X	P	N	N	PE	N	P	N	N	N	N	P	PE	N	*	P*	P*
		6421	T-7E2	X	X	P	PE	PE	N	N	N	N	N	N	N	N	N	N	N	*	N
		6460	T-7E2	X	X	N	P	N	N	P	P	N	N	N	N	P	N	N	*	*	*
R5	2	6406	T-7I0	P*	P*	X	P	P	P	P	P	P	P	P	P	P	N	P*	P*	P*	
		6456	T-7I0	P*	P	X	P	P	P	P	P	P	P	P	P	P	N	P*	P*	P*	
		6435	T-7E2	X	X	P	P	P	PE	P	P	P	P	P	P	P	N	P*	*	P*	

	6451	T-7E2	X	X	N	P	PE	N	N	N	N	P	P	P	N	N	*	*	*
	6458	T-7E2	X	X	P	P	N	PE	PE	PE	P	P	P	P	P	N	P*	P*	P*
	6398	T0I0	P*	P*	X	P*	P	P	P	P	P	P	X	X	X	N	P	P	N
	6444	T0I0	P	P	X	P	P	P	P	P	P	P	N	P	N	P	P*	P*	
1	6420	T0E2	X	X	P	P	P	P	P	P	P	P	N	P	N	P	P*	P*	
	6448	T0E2	X	X	P	P	P	P	N	P	P	P	P	P	P*	P*	P	P*	
	6459	T0E2	X	X	N	P	P	P	PE	N	P	P	N	P	N	N	N	P	
R6	6416	T0I0	P*	P	X	P*	P	P	P	P	P	P	P	P	N	P	P*	P*	
	6461	T0I0	P	P	X	P	P*	P	P	P	P	P	P	P	P*	P	P	P*	
2	6438	T0E2	X	X	N	P	P	P	P	P	P	P	P	P	N	P	N	P	
	6452	T0E2	X	X	N	PE	P	P	P	P	N	P	P	P	P*	P*	N	P	
	6453	T0E2	X	X	N	N	N	N	P	N	P	N	N	N	N	N	N	N	

P: positive result, PE: positive only after enrichment, N: negative result, X: no sample,

*sample positive by PCR for *mcr-1* (for faecal samples, PCR was performed on DNA prepared from faecal samples; for PM samples, PCR was performed on enrichment broths). Absence of asterisk means negative *mcr-1* PCR result.

Grey cells: samples positive according to at least one method

Table 2: Mean daily titres of faecal samples on rifampicin-supplemented media

Room	Group														Positive samples
	(n)	D1**	D2 H0***	D2 H4***	D3	D4	D5	D6	D7	D8	D10	D13	D17	D20	
R4	NTI0 (4)	6.4	5.3		4.7	4.5	4.3	3.9	4.1	3.9	3.8	3.7	3.8	3.9	48/48
	NTE2 (6)			1.75	2.4	2.4	1.7	1.7	2.6	2.8	1.9	2.6	2.2	2.1	53/66
R5	T-7I0 (4)	5.9	5.3		4.7	3.7	3.8	3.6	3.7	3.7	3.0	3.2	3.2	3.6	47/48
	T-7E2 (6)			1.99	2.2	0.7	0.5	0.8	1.2	0.7	1.2	1.3	2.0	1.0	38/66
R6	T0I0 (4)*	5.8	5.1		5.1	4.5	4.1	3.3	3.7	3.8	3.7	3.6	2.6	4.5	44/45
	T0E2 (6)			1.01	2.3	2.6	2.1	2.8	1.6	2.5	1.6	2.1	1.4	2.7	49/66

*One pig in the T0I0 group was euthanised on Day 10

**Day 1

***Day 2, just before (H0) or 4 hours (H4) after transfer into rooms containing inoculated pigs

Table 3: Post-mortem samples: culture on rifampicin-supplemented media

Room	Group	Jejunum	Ileum	Caecum	Colon	Total
R4	NTI0	0/4*	4/4	4/4	4/4	12/16
	NTE2	0/6	2/6	3/6	5/6	10/24
R5	T-7I0	0/4	3/4	3/4	4/4	10/16
	T-7E2	0/6	2/6	2/6	3/6	7/24
R6	T0I0**	1/4	4/4	4/4	3/4	12/16
	T0E2	2/6	4/6	2/6	5/6	13/24
Total		3/30	19/30	18/30	24/30	64/120

The pigs were euthanised sacrificed on Day 20 (pigs NTNI, T-7NI, T0I0 and T0E2), Day 21 (pigs T-7I0 and T-7E2) and Day 22 (pigs NTI0 and NTE2). All samples from non-inoculated pigs were negative.

*number of positive samples/number of tested samples

**One pig in the T0I0 group was euthanised sacrificed on Day 10.

Table 4: *mcr-1* PCR results on enrichment broths from post-mortem samples

Room	Group	Jejunum	Ileum	Caecum	Colon	Total
R4	NTI0	0/4*	3/4	1/4	4/4	8/16
	NTE2	0/6	1/6	3/6	5/6	9/24
R5	T-7I0	0/4	4/4	4/4	4/4	12/16
	T-7E2	0/6	5/6	6/6	5/6	16/24
R6	T0I0**	1/4	0/4	2/4	3/4	6/16
	T0E2	2/6	2/6	1/6	2/6	7/24
Total		3/30	15/30	17/30	23/30	58/120

The pigs were euthanised on Day 20 (pigs NTNI, T-7NI, T0I0 and T0E2), Day 21 (pigs T-7I0 and T-7E2) and Day 22 (pigs NTI0 and NTE2). All samples from non-inoculated pigs were negative.

*number of positive samples/number of tested samples

**One pig in the T0I0 group was euthanised sacrificed on Day 10