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Prevalence of enteric pathogens in diarrheic and non-diarrheic samples from pig farms with neonatal diarrhea in the North East of Spain

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Abstract

Diarrhea is one of the major causes of neonatal mortality in pigs. In the present study, 31 pig farms with outbreaks of neonatal diarrhea were investigated in Catalonia (NE Spain) from February 2017 until June 2018. Two hundred and fifteen diarrheic samples from 1 to 7 days old piglets were tested for a panel of enteric pathogens. In 19 of the studied farms additional fecal samples from apparently healthy pen-mates were collected and tested for the same panel of infectious agents. Samples were bacteriologically cultured and tested by PCR for *E. coli* virulence factors genes, *C. perfringens* types A and C toxins (Cpα, Cpβ, Cpβ2) and *C. difficile* toxins (TcdA, TcdB). Moreover, Rotavirus A (RVA), Rotavirus B (RVB), Rotavirus C (RVC), porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV) were also determined by RT-qPCR. More than one pathogen could be detected in all of the outbreaks. Nevertheless, RVA was the only agent that could be statistically correlated with the outcome of diarrhea. For the other viruses and bacteria analyzed significant differences between the diseased pigs and the controls were not found. In spite of this, the individual analysis of each of the studied farms indicated that other agents such as RVB, RVC, toxigenic *C. difficile* or pathogenic *E. coli* could play a relevant role in the outbreak of diarrhea. In conclusion, the large diversity of agent combinations and disease situations detected in neonatal diarrhea outbreaks of this study stand for a more personalized diagnosis and management advice at a farm level.

Keywords: neonatal diarrhea; pigs; rotavirus; coronavirus; bacterial enteric pathogens

Introduction

Neonatal diarrhea is one of the most frequently disease in modern swine production, which can be associated with high mortality, decreased growth rates and increase of treatment costs (Sjölund *et al*., 2014). Infectious and non-infectious factors can be involved in diarrhea outbreaks in suckling piglets. Among non-infectious factors **stress, poor husbandry and nutrition** can contribute to an animal's susceptibility to disease. Moreover, enteric outbreaks

50 are usually associated to the presence of infectious agents, such as viruses, bacteria or
51 coccidian, although the presence of pathogens in piglets alone does not determine the
52 occurrence of diarrhea episodes (Ruiz *et al,* 2016). All those pathogens can act as primary
53 and sole agents of scours in piglets although co-infections are commonly reported (Kongsted
54 *et al.,* 2018; Mesonero-Escuredo *et al.,* 2018).

55 In recent years, viruses -particularly coronaviruses and rotaviruses- have regained attention
56 as agents of diarrhea in pigs. In regards to rotaviruses, although several genogroups (A, B,
57 C, E and H) have been associated with porcine diarrhea, rotavirus A (RVA) is the most
58 frequent (Marthaler *et al*., 2014). Other species such as rotavirus B (RVB) and C (RVC) have
59 been identified less commonly in diarrheal outbreaks (Morin *et al*., 1990; Martella *et al*.,
60 2007, Amimo *et al*., 2013b). RVB has been reported in several Asian countries, North
61 America, South Africa and Brazil, but rarely in Europe (Smitalova *et al*., 2009; Otto *et al*.,
62 2015). However, there are still few studies conducted on RVB, RVC and other genogroups
63 to determine their importance in porcine diarrhea outbreaks.

64 Regarding porcine coronaviruses, transmissible gastroenteritis virus (TGEV) and porcine
65 epidemic diarrhea virus (PEDV) can also cause diarrheal outbreaks with high morbidity and
66 mortality in neonatal pigs. However, since 2014 the most recent outbreaks in Europe have
67 been related with PEDV (Carvajal *et al*., 2015, Laranjo *et al,* 2015).

68 As regards bacterial infections, *Escherichia coli* (*E. coli*) has historically been considered
69 one of the main agents causing neonatal diarrhea in pigs (Chan *et al.*, 2013). Different *E. coli*
70 pathotypes have been identified based on toxin production and other virulence factors. The
71 most common are the enterotoxigenic *E. coli* (ETEC) strains, characterized by the production
72 of enterotoxins (STa, STb and LT). Other pathotypes of *E.coli* have been detected in piglets,
73 such as enteropathogenic *E. coli* (EPEC) strains, producing intimin (*eae* gene), although less
74 frequently. (Toledo *et al.*, 2012). Anaerobic bacterial pathogens such as enterotoxigenic

75 strains of *Clostridium perfringens* (*C. perfringens)* type A (producing Cpα toxin), *C.*
76 *perfringens* type C (producing Cpα and Cpβ toxins) and *Clostridium difficile (C. difficile)*
77 producing enterotoxin A (TcdA) and/or cytotoxin B (TcdB) have also been reported in
78 diseased piglets (Uzal and Songer, 2019).

79 Ideally, diagnosis of outbreaks of neonatal diarrhea should consider the clinical findings and
80 lesions, the epidemiological pattern and the detection of the infectious agents potentially
81 involved. However, most often the diagnosis of enteric diseases is mainly focused on some
82 predominant infectious agents. Yet, in most cases, several agents with the potential for
83 producing diarrhea in piglets are found in the same outbreak.

84 The objective of this study was to determine the prevalence of several pathogens related with
85 neonatal diarrhea and to compare their frequencies with that of healthy penmates in a
86 framework of diagnostic analysis.

87 Materials and methods

88 Sampling

89 Thirty-one conventional farrow-to-finish farms presenting neonatal diarrhea in piglets aged
90 between 1 and 7 days were included in the study. In each farm, 10 samples from diarrheic
91 animals and 5 samples from apparently healthy penmates were asked to be collected. Fecal
92 samples were submitted for diagnostic to the Laboratori Veterinari de Diagnosi de Malalties
93 Infeccioses, of the Universitat Autònoma de Barcelona (Spain), between February 2017 and
94 June 2018. Farms were located in Catalonia (NE of Spain), one of the regions of Europe with
95 a higher pig density (242 pigs/km^2). Finally, a total of 215 diarrheic samples were taken from
96 the 31 tested farms (5-10 animals/farm). Additionally, from 19 of these studied farms, 88
97 fecal samples (3-5 animals/farm) were obtained from apparently healthy pen-mates that did
98 not present diarrhea at the moment of sampling. One gram of fecal Sample was obtained

99 directly from the animals using rectal swabs. A farm was considered to be positive for a
100 specific pathogen when at least one sample of the tested animals was found positive for that
101 pathogen.

102 Microbiological testing

103 Stool samples were directly analyzed upon arrival for microbiological identification of *E.*
104 *coli, C. perfringens* and *C. difficile* and an aliquot of each sample was stored at -80 °C.

105 For *E. coli* isolation, samples were aerobically cultured on Columbia blood agar (BD GmBh,
106 Germany) and MacConkey agar plates (Oxoid, UK), and were incubated during 24 hours at
107 37 °C.

108 To recover *C. perfringens* and *C. difficile* from faeces, samples were firstly treated with
109 ethanol (96%) 35 min to eliminate the vegetative cells and then centrifuged (x 8,000 g) as
110 described by Koransky *et al.* (1978). The pellet was then cultured on a selective medium
111 *Clostridium difficile* agar base (Conda Laboratorios, Spain), and incubated anaerobically for
112 48 hours at 37 °C.

113 Molecular diagnosis of viral agents

114 Faecal samples were centrifuged (6,000 g, 5 min) before the RNA extraction. Non-diarrheic
115 samples were initially diluted in 500 μL of sterile distilled water before centrifuging. The
116 Nucleospin RNA extraction kit (Macherey-Nagel, Germany) was used following the
117 manufacturer’s instructions. The final extracted RNA was suspended in 50 μL of RNAse-
118 free water (Macherey-Nagel, Germany). Detection of viral agents was done using the
119 AgPath-ID™ One-Step RT-PCR kit (Applied Biosystems, ThermoFisher, USA). For RVA,
120 PEDV and TGEV, the protocol designed by Masuda *et al*. (2016) was followed, and RVB
121 and RVC were detected using a previously described RT-PCR by Marthaler *et al*. (2014).

122 Molecular diagnosis of bacterial agents

123 DNA was extracted from bacterial cultures by boiling. Briefly, all bacterial growth from
124 MacConkey plates and *Clostridium* spp. selective medium plates from all samples were
125 diluted in 600 µL of sterile distilled water and 200 µL of the dilution were then transferred
126 to a new tube. Two-hundred microliters of sterile distilled water were added to each tube.
127 Tubes were boiled in a water bath for 10 min, and then centrifuged at 13,000 rpm for 5 min.
128 After centrifugation, the supernatant was recovered and stored at -80 °C until processed.

129 The presence of *E. coli* adhesins (F4, F5, F6, F18, F41 and *eae*) and toxins (LT, Sta, STb,
130 EAST1) was analysed using conventional PCR. VT1 and VT2 toxins were included as a
131 routine basis in this general diagnostic panel of *E. coli*. . *C. perfringens* (α, β and β2) and *C.*
132 *difficile* (TcdA and TcdB) toxins were also evaluated by PCR.

133 For all PCR, the master mix consisted of: 1x PCR Buffer, 0.2 mM of each dNTP (Bioline,
134 France), 3 mM of MgCl2, 1 mM of each primer and 1 U of Taq DNA Polymerase (Bioline,
135 France). A final volume of 2.5 µL of DNA was used in the PCR. In each reaction, positive
136 and negative controls were included.

137 The characterisation of *E.coli*, as regards to the presence of adhesins and *eae*) and toxins was
138 done using the primers described by Toledo *et al*. (2012). The PCR program consisted of 5
139 min at 94ºC, followed by 30 cycles of 1 min at 94ºC, 1 min of annealing at 63ºC and 1 min
140 of extension at 72º, and a final extension step of 7 min at 72ºC.

141 The detection of toxigenic *C. difficile* strains was done by a standardized PCR protocol for
142 TcdA and TcdB previously described by Persson *et al*. (2008). The PCR program consisted
143 of 10 min at 94 ºC, followed by 25 cycles of 50 s at 94ºC, 40 s of annealing at 53ºC and 50 s
144 of extension at 72ºC, and a final extension step of 3 min at 72ºC. For the detection of *C.*
145 *perfringens* type A and C, specific PCR were carried out using the primers described by van
146 Asten *et al.* (2009), and the program consisted of 5 min at 95ºC, followed by 30 cycles of 1
147 min at 94ºC, 1 min annealing at 53ºC and 1 min of extension at 72ºC, and a final extension
148 step of 10 min at 72ºC.

149

150 Reference *E. coli* and *C. perfringens* strains used as positive controls were kindly donated by
151 Dr. Blanco (*E. coli* Reference Laboratory, Santiago de Compostela, Spain). Positive *C.*
152 *difficile* strain was kindly provided by Dr. Sanfeliu (UDIAT Diagnostic Centre, Sabadell,
153 Spain).

154 PCR products were resolved in a 1.5 % agarose gel by electrophoresis. Reference positive
155 strains and a 100 bp ladder (Biotools B&M labs, Spain) were used to identify the positive
156 samples. Amplified PCR products were visualized using ethidium bromide staining under
157 UV light.

158

159 Results

160 Overall prevalence of enteric pathogens in the diarrheic outbreaks

161 The prevalence of enteric pathogens from the diarrheic cases evaluated in the study showed
162 a high diversity in the proportion of positive samples per each of the 31 farm tested (Figure
163 1). *C. perfringens* A, *C. difficile* toxigenic strains, and RVA and RVC were the most
164 frequently agents diagnosed at farm level (Figure 1).

165 Regarding the number of diarrheic animals in the overall population analyzed, viruses
166 presented the following prevalence (Table 1): 51.6% (111/215) of samples were positive for
167 RVA, 9.3% (20/215) for RVB and 39.1% (84/215) for RVC. The percentage of samples
168 positive to coronaviruses was low (11/215, 5.1%), 6 of them being positive to TGEV and 5
169 to PEDV. Regarding bacterial agents, *C. perfringens* Cpα toxin was found in 71% (152/215)
170 of the samples while only 7 samples (3.3%) from two different farms were found positive for
171 Cpβ toxin (Table 1). Moreover, Cpβ2 toxin was detected in 87% of Cpα toxin positive

172 samples (132/152). TcdA and TcdB *C. difficile* isolates were found in 28.9% (62/215) and
173 34% (73/215) of the samples, respectively, 22.3% of the samples being TcdA/TcdB double
174 positive.

175 *E. coli* was isolated in pure culture in 44% (94/215) of the tested samples from diarrheic
176 animals. The virulence factor characterization of these 94 isolates showed a low prevalence
177 (<5%) of *E. coli* toxins and fimbriae, except for STb (10.7%), *eae* (9.8%) and EAST1 (56%)
178 genes (Table 2). *E. coli* strains that could be classified into a pathotype were isolated from
179 21/31 (67.7%) farms but with a low proportion of positive samples. The highest prevalence
180 corresponded to the ETEC pathotype (12.1%), harboring STa, STb and/or LT genes,
181 followed by the EPEC pathotype (9.8%) with the *eae* gene, and lasting with an occasional
182 VTEC (5.1%) strains, none of them harboring neither VT1 and VT2 genes.

183 Prevalence and combination of enteric pathogens at farm level

184 Rotaviruses were detected in 30 out of 31 farms (Table 3). RVA (80.6%) and RVC (71%)
185 were isolated from most of the farms (25 and 22, respectively), and were detected
186 concomitantly in 17 of them (54.8%). By contrast, only 7 farms were positive to RVB, always
187 found in co-infection with RVC. Finally, PEDV and TGEV were detected in 4 and 3 farms
188 respectively.

189 As regards the bacterial agents, *C. perfringens* A was found in 100% of farms, followed by
190 *C. difficile* toxigenic strains (87.1% farms). Pathogenic *E.coli*, mainly ETEC and EPEC
191 strains, was found in 64.5% of farms. Finally, 58% of farms were positive to RVA, RVC,
192 *C.difficile* and CpA co-infection (Table3).

193 Comparison of results between diarrheic and healthy piglets

194 Diarrheic animals (n=140) and non-diarrheic (n=88) penmates were sampled in 19 farms.
195 RVA was the only pathogen statistically associated with the cases of diarrhea [61.4% vs

196 31.8%, p<0.001] (Table 4). Regarding bacterial pathogens no statistical differences were
197 found when comparing diseased versus non-diseased pen-mates although prevalence of *C.*
198 *difficile* TcdA and *E. coli* F41 or STa toxigenic strains were slightly higher in the diseased
199 animals (Table 4).

200 There were 6/19 farms in which RVA could not have a prominent role in the diarrhea
201 outbreak, either because of the absence of RVA positive animals in the farm or because the
202 RVA prevalence was higher in healthy animals than in diseased cases. In those particular
203 cases, other bacterial agents such as toxigenic *C. difficile* (TcdA / TcdB) or pathogenic *E.*
204 *coli* (mainly ETEC or VTEC), or other viruses, such as RVB, could be identified in a larger
205 proportion of diseased pigs compared to the healthy pen-mates (Figure 2).

206 Discussion

207 The present study reports data of the prevalence of the main pathogens associated with
208 neonatal diarrhea in Spain. In general, *C. perfringens* type A, toxigenic *C. difficile* and
209 rotavirus could be isolated from most of the analyzed farms. Furthermore, most of the
210 analyzed samples of this study, regardless the health status of the piglet, were positive for
211 multiple combinations of pathogens, involving principally RVA, RVC, *E. coli*, and toxigenic
212 strains of *C. difficile* and *C. perfringens* type A. Although some of the animals considered
213 healthy could be incubating infections, it seems unlikely due to the short incubation period
214 of enteric infections. Moreover, the large number of combinations of pathogens, possibly
215 along with other non-infectious causes, stands for the multifactorial origin of the neonatal
216 diarrhea in pigs and shows the actual complexity of this condition.

217 One of the main results of this study is the evidence of RVA as the most frequent agent
218 involved in neonatal diarrhea of the studied cases. This result agrees with a recent case-
219 control study conducted in pig farms in Denmark, in which the authors concluded that RVA

220 was the only agent that could be statistically associated to neonatal diarrhea (Kongsted *et al.,*
221 2018)**.** Nevertheless in some cases their role as a causative agent of disease in pigs have been
222 controversial. While some studies did not find a clear association between RVA infection
223 and neonatal disease (Ruiz *et al.,* 2016; Amimo *et al.,* 2013a), others did find a statistical
224 relationship between neonatal diarrhea and RVA single infection (Linares *et al.,* 2009;
225 Kongsted *et al.,* 2018; Mesonero-Escuredo *et al.,* 2018), or RVA combined with other factors
226 such as co-infections or management conditions (Ruiz *et al.* 2016).

227 As regards to other rotaviruses, most of the RVC positive farms found in this study were also
228 positive to RVA, and had similar frequencies of RVC positive animals in piglets suffering
229 diarrhea and in the healthy penmates. A recent study made in Danish pigs affected by the
230 new neonatal porcine diarrhea syndrome (NNPDS) found that regarding rotaviruses only 9%
231 of pigs were positive to RVA and none to RVC by RT-qPCR (Goecke et al. 2017). By
232 contrast, for RVB, differences in the frequency of this pathogen in diseased and healthy
233 piglets were clearer although the global RVB prevalence was low. Thus, in the context of our
234 study, both RVA and RVB could be considered as important causing agents of diarrhea in
235 some outbreaks.

236 The number of papers on the prevalence of RVB and RVC in pigs is relatively scarce. In a
237 study conducted in the United States, the authors reported similar rotaviruses prevalence with
238 62% RVA**,** followed by 53% RVC and 33% RVB (Marthaler *et al.,* 2014). Rotavirus B has
239 also been detected in several Asian countries, South Africa, and Brazil (Alekseev *et al.,*
240 2015). In Europe, limited reports of RVB have been described in Germany (Otto *et al.,* 2015)
241 and the Czech Republic (Smitalova *et al.,* 2009) so far, with prevalence of 1.6% and 0.6%
242 respectively. The differences in the obtained prevalence between those studies and the
243 present work could be explained by the study design, the age of the animals or the
244 geographical area of sampling.

245 Regarding the analyzed coronaviruses, PEDV and TGEV, only 13 animals from 8 different
246 farms were positive. Similar results were found recently in Spain by Mesonero-Escuredo *et*
247 *al*. (2018), who reported a 3.7% prevalence for PEDV. Regarding the positive samples to
248 TGEV in the present study it must be mentioned that the PCR that we used could not
249 distinguish TGEV from porcine respiratory coronavirus (PRCV) since the target gene was
250 the viral nucleocapside. Besides this, the spread of PRCV across Europe since its emergence
251 in the 1980 decade reduced the prevalence of TGEV because of the crossed immunity
252 between the two viruses. Nowadays diarrhea caused by TGEV is uncommon in Europe (Saif
253 *et al*., 2009).

254 The prevalence of *C. perfringens* type A, as well as the Cpβ2 positive strains, was very high
255 and similar between diarrheic and healthy pigs. The role of the cpβ2 toxin in the pathogenesis
256 of neonatal diarrhea is controversial and while some studies have associated it with diarrhea
257 outbreaks (Garmory *et al.,* 2000; Bueschel *et al.,* 2003), others found no differences between
258 diseased and healthy pigs (Jaggi *et al.,* 2009; Farzan *et al.,* 2013; Lee *et al.,* 2014). Since *C.*
259 *perfringens* type A is a common gut microorganism, the detection of this agent cannot be
260 interpreted unambiguously, given that it is not possible to distinguish commensal from
261 pathogenic strains. Thus, although it has been considered as a main pathogen involved in
262 persistent neonatal diarrhea (Mesonero-Escuredo *et al.,* 2018), a direct pathogen-toxin-
263 disease association has not been yet determined (Kongsted et al., 2013, Kongsted et al.,
264 2018). As regards *C. perfringens* type C, the prevalence detected in the present study was
265 low. This could be the result of the routine vaccination plan implemented in sows (Salvarani
266 *et al.,* 2013).

267 It has been suggested that *C. difficile* could be one of the most important uncontrolled cause
268 of neonatal diarrhea in pigs in some scenarios (Songer and Anderson*.,* 2006) with
269 significantly higher prevalence in diarrheic piglets (Kim *et al.,* 2018). However, in our study,

270 the general prevalence of toxigenic *C. difficile* was similar in both healthy and diarrheic
271 animals. Other studies have reported a high prevalence of *C. difficile* toxins in healthy
272 animals concluding no clear relationship between diarrheal outbreaks and the detection of
273 toxigenic *C. difficile* in pigs (Yaeger *et al.,* 2007, Álvarez-Pérez *et al.,* 2009).

274 ETEC has been and still is considered the main agent responsible for intestinal disorders in
275 neonatal piglets **b**eing F4, F5, F6 and F41, the main fimbriae associated with diarrhea,
276 (Dubreuil *et al.,* 2016; Luppi *et al.,* 2016). In the present study, ETEC strains were
277 infrequently isolated from both diarrheic and non-diarrheic piglets, similar to the results
278 reported previously by others (Kongsted et al., 2013; Kongsted et al., 2018, Larsson *et al.,*
279 2015; Mesonero-Escuredo *et al.,* 2018; Toledo *et al.* 2012). This low prevalence of *E. coli*
280 pathotypes and virulent factors is probably related to the *E. coli* vaccination programs
281 implemented in sows in the Spanish farms. Most of these vaccines available on the market
282 contain *E. coli* fimbriae (mostly F4, F5, F6 and F41) and toxoids (such as LT), and therefore,
283 prevent the infection caused by pathogenic strains of *E. coli*. By contrast, EAST1 positive *E.*
284 *coli* were very common. The pathogenic role of the EAST1 toxin is not clear, given that it
285 has also been found in a high prevalence in strains from healthy animals of our study in
286 agreement with the results published by Zajacova *et al.*(2012).Nevertheless, in some farms
287 in which RVA was not considered to be the main causative agent, the diarrheic process of
288 the piglets could be associated to pathogenic *E. coli* or toxigenic *C. difficile* strains.

289 The high frequency of multiple infections detected in diarrheic and healthy piglets makes
290 the setting up of a final diagnosis a very difficult task. Additional information supplied by
291 complementary techniques or studies, may help to achieve a definitive diagnosis.

292

293 In conclusion, the large diversity of agent combinations and disease situations detected in the
294 different pig farms confirms the multifactorial origin of the neonatal diarrhea in pigs and
295 stand for a more personalized diagnosis and management advice at a farm level, including
296 also non-infectious factors that can trigger neonatal diarrhea.

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301 Conflict of interest statement

302 The authors do not have any conflict of interest.

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472 diarrheic piglets and presence of adhesion involving virulence factors in astA positive strains.
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474

475 Table legends

476 **Table 1.** Prevalence of viral agents and clostridial toxin genes detected by PCR in samples
477 of diarrheic piglets (n=215) from 31 tested farms.

478 **Table 2**. Prevalence of *E. coli* pathotypes, virulence factors and toxins at animal and farm
479 level.

480 **Table 3.** Distribution of farms positive to the different panel of enteric pathogens.
481 RVA/B/C, Rotavirus A/B/C; *C. difficile*, toxigenic strains (TcdA, TcdB); *E. coli*,
482 pathogenic *E.coli;* PCoV, porcine coronaviruses; Cp A/C, *C. perfringens* A/C.

483 **Table 4.** Proportion and statistical values of enteric agents between diarrheic (n=140) and
484 healthy (n=88) animals.

485 Figure legends

486 **Figure 1**. Proportion of positive samples for each analyzed farms (n=31) and enteric
487 pathogens by Boxplot. RVA/B/C, rotavirus A/B/C; PCoV, porcine coronaviruses (PEDV
488 and TGEV); TcdA/B, *C. difficile* toxins; Cpα/β2*, C. perfringens* toxins; ETEC,
489 enterotoxigenic *E. coli;* EPEC, enteropathogenic *E. coli*; VTEC, verotoxigenic *E. coli*.
490 The distribution of data is displayed as follows: the box is determined by the Interquartile
491 Range (IQR: 25th and 75th percentiles) and the median line shows the middle value of
492 the dataset; the whiskers are determined by the 5th and 95th percentiles; minimum and
493 maximum values are shown at the ends of the bars and outliers as gray dots.

494 **Figure 2.** Comparison of prevalence of positive samples between diarrheic (D, black bar)
495 and non-diarrheic groups (ND, light bar) distributed by farms (Fn) and enteric pathogens.
496 RVA/B/C, rotavirus A/B/C; PCoV, porcine coronaviruses (PEDV and TGEV); TcdA/B,

497 *C. difficile* toxins; Cpα/β2*, C. perfringens* toxins; ETEC, enterotoxigenic *E. coli;* EPEC,

498 enteropathogenic *E. coli*; VTEC, verotoxigenic *E. coli*.

	Piglets (N=215)		Farms (N=31)	
Pathogen	Number	%	Number	%
Viral agents				
RVA	111	51.6	25	80.6
RVB	20	9.3	7	22.6
RVC	84	39.1	22	71
PCoV	11	5.1	7	22.6
C. perfringens				
Cpα	152	70.7	31	100
Cpβ	7	3.3	2	6.4
Cpβ_2	132	61.4	30	96.8
C. difficile				
TcdA	62	28.9	25	80.6
TcdB	73	34	25	80.6

Pathotype	Adhesins	Toxins	Pigs		Farms	
			n	%	n	%
ETEC	F4	STa, STb	2	1	1	3.2
	ND	LT	2	1	1	3.2
		LT, STb	1	0.5	1	3.2
		STa, STb	1	0.5	1	3.2
		STa	1	0.5	1	3.2
		STb	12	5.6	6	19.4
EPEC	F18, eae	ND	1	0.5	1	3.2
	F41, eae		2	1	2	6.5
	eae		14	6.5	8	25.8
VTEC	ND	VT1	3	1.5	3	9.7
ETEC/EPEC	F41, eae	STb	1	0.5	1	3.2
ETEC/VTEC	F4	STa, STb, VT2	1	0.5	1	3.2
	ND	VT2, STb	5	2.3	3	9.7
EPEC/VTEC	eae	VT1	3	1.5	1	3.2

ND: not detected