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

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24 Abstract

25   Diarrhea is one of the major causes of neonatal mortality in pigs. In the present study, 31 pig

26   farms with outbreaks of neonatal diarrhea were investigated in Catalonia (NE Spain) from

27   February 2017 until June 2018. Two hundred and fifteen diarrheic samples from 1 to 7 days

28   old piglets were tested for a panel of enteric pathogens. In 19 of the studied farms additional

29   fecal samples from apparently healthy pen-mates were collected and tested for the same panel

30   of infectious agents. Samples were bacteriologically cultured and tested by PCR for *E. coli*

31   virulence factors genes, *C. perfringens* types A and C toxins (Cpα, Cpβ, Cpβ2) and *C. difficile*

32   toxins (TcdA, TcdB). Moreover, Rotavirus A (RVA), Rotavirus B (RVB), Rotavirus C

33   (RVC), porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus

34   (TGEV) were also determined by RT-qPCR. More than one pathogen could be detected in

35   all of the outbreaks. Nevertheless, RVA was the only agent that could be statistically

36   correlated with the outcome of diarrhea. For the other viruses and bacteria analyzed

37   significant differences between the diseased pigs and the controls were not found. In spite of

38   this, the individual analysis of each of the studied farms indicated that other agents such as

39   RVB, RVC, toxigenic *C. difficile* or pathogenic *E. coli* could play a relevant role in the

40   outbreak of diarrhea. In conclusion, the large diversity of agent combinations and disease

41   situations detected in neonatal diarrhea outbreaks of this study stand for a more personalized

42   diagnosis and management advice at a farm level.

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

44   Introduction

45   Neonatal diarrhea is one of the most frequently disease in modern swine production, which

46   can be associated with high mortality, decreased growth rates and increase of treatment costs

47   (Sjölund *et al*., 2014). Infectious and non-infectious factors can be involved in diarrhea

48   outbreaks in suckling piglets. Among non-infectious factors **stress, poor husbandry and**

49   **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

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

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

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

Materials and methods

Sampling

Thirty-one conventional farrow-to-finish farms presenting neonatal diarrhea in piglets aged between 1 and 7 days were included in the study. In each farm, 10 samples from diarrheic animals and 5 samples from apparently healthy penmates were asked to be collected. Fecal samples were submitted for diagnostic to the Laboratori Veterinari de Diagnosi de Malalties Infeccioses, of the Universitat Autònoma de Barcelona (Spain), between February 2017 and June 2018. Farms were located in Catalonia (NE of Spain), one of the regions of Europe with a higher pig density (242 pigs/km^2). Finally, a total of 215 diarrheic samples were taken from the 31 tested farms (5-10 animals/farm). Additionally, from 19 of these studied farms, 88 fecal samples (3-5 animals/farm) were obtained from apparently healthy pen-mates that did 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 $MgCl_2$, 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.

301   Conflict of interest statement

302   The authors do not have any conflict of interest.

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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,

19

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

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

Pathogen	Piglets (N=215)		Farms (N=31)	
	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