Experimental infection of Specific-Pathogen-Free pigs with: excretion in faeces and transmission to non-inoculated pigs

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1 **Experimental infection of Specific-Pathogen-Free pigs with**
2 ***Campylobacter*: excretion in faeces and transmission to non-**
3 **inoculated pigs**

4
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21

1 **Abstract**

2

3 *Campylobacter* species are leading agents of human bacterial gastroenteritis and
4 consumption of food of animal origin is a major source of infection. Although pigs are
5 known to frequently exhibit high counts of *Campylobacter* in their faeces, more
6 information is needed about the dynamics of this excretion. An experimental trial was
7 conducted to evaluate the faecal excretion of *Campylobacter* by 7-week-old specific
8 pathogen-free piglets inoculated *per os* with three *Campylobacter* strains (one *C. coli*
9 isolated from a pig, one *C. coli* and one *C. jejuni* from chickens) alone or simultaneously
10 (5×10^7 CFU per strain). Non-inoculated pigs were housed in adjacent pens. Pigs were
11 monitored for 80 days for clinical signs and by bacteriological analysis of faeces. Pigs
12 inoculated with porcine *C. coli* or with a mix of the three strains excreted from 10^3 to 10^6
13 CFU/g of faeces with a slight decrease at the end of the trial. Animals inoculated with
14 poultry *C. coli* or *C. jejuni* strain excreted a lower quantity and some of them stopped
15 excreting. At the end of the trial, only *C. coli* was detected in the faeces of pigs inoculated
16 simultaneously with the three bacteria. Moreover, the transmission of *Campylobacter* was
17 noticed between pens for the two *C. coli* strains and all the neighbouring animals became
18 shedders with a level of excretion similar to the inoculated pigs. Intermittence in the
19 *Campylobacter* excretion was also observed. Finally, our study highlighted a host
20 preference of *Campylobacter*, namely *C. coli* seems to have a higher colonization
21 potential for pigs than *C. jejuni*.

22

23 **Keywords**

24 *Campylobacter*, Pigs, Faecal excretion, Experimental infection

1 **1 INTRODUCTION**

2 *Campylobacter*, a major cause of food-borne gastro-enteritis, is commonly carried in the
3 intestinal tract of a wide range of birds and mammals, including food production animals
4 and pets, without causing clinical signs (Petersen et al., 2001; Moore et al., 2005; Englen
5 et al., 2007; Keller et al., 2007). Recently, the 2003/99/CE directive on the monitoring of
6 zoonoses and zoonotic pathogens has forced the European Union Member States to
7 collect relevant and comparable data on these agents. Pigs seem to be a natural reservoir
8 of *Campylobacter* species with a prevalence of infection between 50 and 100% and
9 excretion levels ranging from 10^2 to 10^7 bacteria per gram of faeces (Nielsen et al., 1997;
10 Weijtens et al., 1997; Von Altröck et al., 2006). Variable counts of *Campylobacter* in the
11 faeces were observed and a possible intermittent excretion was suggested by Weijtens et
12 al. (1999). *Campylobacter coli* has been shown to be the predominant species carried by
13 pigs (Payot et al., 2004; Alter et al., 2005; Thakur and Gebreyes, 2005). Nevertheless,
14 one study found a high prevalence of *C. jejuni* on an American farm (Young et al., 2000)
15 and *C. jejuni* may co-exist with *C. coli* in pigs (Madden et al., 2000; Jensen et al., 2005).
16 However, these findings were based on small sample sizes and often concerned only one
17 or few farms with occasional sampling schemes. Better knowledge of the dynamics of
18 excretion of *Campylobacter* in pig faeces is thus necessary to understand the way of
19 transmission and dissemination of this bacterium.

20 The aims of the present study were to describe *Campylobacter* excretion in pigs under
21 controlled conditions after experimental infection of SPF (Specific Pathogen Free) piglets
22 and to assess the possible transmission to pigs housed in adjacent pens. The preferential
23 infection of pigs in the field with *Campylobacter coli* suggests a differential capacity of
24 *Campylobacter* species/strains to successfully colonize the digestive tract of pigs. To
25 assess this, the experimental infection was carried out using *C. coli* strains of two origins

1 (poultry and porcine) and *C. jejuni*. Furthermore, we were interested in studying the
2 possible co-existence of different *Campylobacter* strains and/or species in pigs. The
3 experiment thus included animals inoculated with the three strains in a mix.

2 MATERIAL AND METHODS

2.1 *Campylobacter* strains and inoculum preparation

Three *Campylobacter* field strains, stored at -80°C in glycerol peptone broth, were used in this experiment. One *C. coli* strain was isolated from faeces of pigs collected in a French slaughterhouse. The other two strains, one *C. coli* and one *C. jejuni*, were obtained from caeca of standard broilers from two different French slaughterhouses. These strains will be hereafter referred to as “porcine *C. coli*”, “poultry *C. coli*” and *C. jejuni* respectively. These strains were differentiated by their Pulsed Field Gel Electrophoresis (PFGE) patterns.

10

For pig inoculation, *Campylobacter* culture was performed on Karmali plates in a micro-aerophilic atmosphere (7% O₂, 10% CO₂, 83% N₂) for 24 hours at a temperature of 41.5°C. Colonies were suspended in 50 mL of sterile Brucella broth and cultivated for 16 hours in the same conditions as above in order to obtain in broth a solution of 5 10⁸ CFU of *Campylobacter*/ml for each strain. One ml of each culture was diluted in 9 ml of tryptone salt medium in order to obtain an inoculum for a pig of 10 ml containing 5 10⁷ CFU of *Campylobacter*.

18

2.2 Animal samples and experimental design of the trial

Specific-pathogen-free (SPF) 7-week-old Large White piglets were obtained from the high-security barn at the French Agency of Food Safety located in Ploufragan (France). Pigs were housed and treated in accordance with the regulations of the local veterinary office (Direction des Services Vétérinaires des Côtes d’Armor, France). All the animals were reared in isolation rooms with controlled air flow and the experiment was carried out in standardized conditions (Cariolet et al., 2004).

1 Twenty-one SPF piglets were distributed into 7 groups of three animals (Figure 1). One
 2 group of three piglets was kept as negative controls and placed in a separate unit (one unit
 3 corresponding to one room) (Unit number 4, Pen g). The 18 other piglets were placed in
 4 three separate units (Unit numbers 1, 2 and 3) each including two pens with 3 animals
 5 each. In Unit 1, three pigs in Pen a were orally inoculated each with 10 mL of tryptone
 6 salt medium containing 5×10^7 CFU of porcine *Campylobacter coli*. In Pen b, three animals
 7 received the same volume of tryptone salt medium without bacteria. The sham inoculated
 8 pigs will be referred to as “neighbouring pigs” hereafter. Unit 2 consisted of a pen of
 9 piglets inoculated each with 5×10^7 CFU of poultry *C. coli* (Pen c) and the second pen of
 10 piglets inoculated each with 5×10^7 CFU of *C. jejuni* (Pen d). Unit 3 included a pen of
 11 piglets inoculated each with a mix of the three stains ($3 \times 5 \times 10^7$ CFU) (Pen e) and a pen
 12 with three neighbouring animals treated with the same volume of tryptone salt medium
 13 without bacteria (Pen f). Finally, strict rules of circulation were established to avoid
 14 transmission of *Campylobacter* between units (clean material for each pen, floor washed
 15 before any sampling, hands and feet washed before and after the sampling). Inside a
 16 given unit, non-inoculated pigs were always manipulated at first. In the Unit 2, *C. jejuni*
 17 inoculated pigs were manipulated before *C. coli* inoculated pigs. Moreover, the animal
 18 handler manipulated the group of piglets kept as negative controls and housed in a
 19 separate unit at the end of each sampling time.

21 **2.3 Clinical examination**

22 Piglets were monitored daily after inoculation to check for clinical signs of disease (rectal
 23 temperature, general inspection), notably diarrhoea. Body weight and food consumption
 24 were measured twice a week to assess growth performances. If clinical signs like fever or
 25 diarrhoea were observed, bacterial investigations were planned in order to determine the

1 etiology. Moreover, in case of mortality, it was planned to carry out a necropsic
 2 examination and to collect samples for bacterial investigations. The pigs were examined
 3 this way for 80 days after inoculation.

5 **2.4 Collection of faecal and environmental samples**

6 Before inoculation, faecal samples and environmental samples from each pen were
 7 analysed to confirm that piglets were free of *Campylobacter*. For the environment, swabs
 8 were collected from each pen and from the floor and walls around each pen in each unit
 9 the day before the inoculation of pigs. Each week, swabs were sampled on the air filter in
 10 each unit to detect *Campylobacter* in the air and feed samples were taken in the feed
 11 storage room. After inoculation, faecal samples of pigs were collected individually once a
 12 week from pigs aged 7 to 16 weeks and were cultured within 4 hours after sampling. At
 13 the end of the experiment, the pigs were slaughtered and the caecal and rectal content of
 14 each animal were collected separately for bacteriological analyses.

16 **2.5 Enumeration and isolation of *Campylobacter* spp.**

17 *Campylobacter* was cultured in a microaerophilic atmosphere (5% O₂, 10% CO₂, 85%
 18 N₂) and at a temperature of 41.5°C. Ten grams of fresh faeces were added to 90 ml of
 19 Preston broth (Oxoid, Dardilly, France) with a Preston antibiotic supplement (AES
 20 laboratory, Rennes, France). For *Campylobacter* numeration, a ten-fold dilution serie (10⁻¹
 21 to 10⁻⁵) was carried out and 100µl of each dilution were plated on Karmali agar (AES
 22 laboratory, Rennes, France) and incubated for 24 hours. A second plating was carried out
 23 24 hours after broth enrichment in order to confirm the absence of *Campylobacter* in the
 24 samples or to confirm the presence of *Campylobacter* in samples harbouring less than
 25 100 CFU of *Campylobacter*/g of faeces (detection limit of the direct method). After 48

1 hours of incubation (72 hours for direct streaking) of the agar plates, presence of
 2 *Campylobacter* colonies was checked and the number of CFU/g of faeces was calculated.
 3 For the non-inoculated pigs, the faecal samples were cultured after an enrichment step of
 4 24 hours to determine the presence or the absence of *Campylobacter*. If successive
 5 positive samples were observed, a *Campylobacter* numeration, like for the inoculated
 6 pigs, was planned. From each positive sample corresponding to one pig, 10 colonies
 7 taken at random were sub-cultured on blood agar for 48h at 41.5°C. The *Campylobacter*
 8 isolate was stored at -80°C in glycerol peptone broth. Random colonies were suspended
 9 in 100 µl TE buffer (10mmol l⁻¹ tris-HCl, 1mmol l⁻¹ EDTA, pH 7.6) and stored at -20°C
 10 for DNA extraction. For each pig, excreted colonies obtained at the end of the trial were
 11 species identified by PCR. If animals did not excrete any more at the last sampling time,
 12 PCR identification was carried out on isolates obtained from previous samples.

14 **2.6 DNA extraction and PCR for species identification**

15 DNA extraction was performed by heating the bacteria at 95°C for 10 min. After a low-
 16 speed centrifugation (5000 x g, 2min), 3µL of the supernatant was used for species
 17 identification by PCR. PCR and electrophoresis were performed as described by Denis et
 18 al. (1999) to distinguish *C. coli* to *C. jejuni* strains. The three inoculated strains were used
 19 as positive controls in the PCR.

21 **2.7 Pulsed Field Gel Electrophoresis (PFGE)**

22 At the end of the trial, 20 isolates (10 from caecal content, 10 from rectal content) per pig
 23 in Unit 3 were genotyped by PFGE as described by Rivoal et al. (2005) in order to
 24 distinguish which strains were excreted, namely to differentiate porcine *C. coli* and

- 1 poultry *C. coli*. Two DNA restriction patterns were obtained for each isolate by using two
- 2 restriction enzymes, *Sma* I and *Kpn* I.

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2 **3 RESULTS**

3 **3.1 Clinical observations and growth performances**

4 Few pigs exhibited clinical signs during the entire trial. Two animals from the group
5 inoculated simultaneously with the three strains (Pen e) had diarrhoea for one day without
6 associated hyperthermia or anorexia. Moreover, post-mortem examination did not reveal
7 any pathological change. The mean average daily gain (ADG) value was calculated for
8 each pen. The results were similar between groups with 1039, 907 and 925g for pigs
9 inoculated with one strain (porcine *C. coli*, poultry *C. coli* and *C. jejuni* respectively) and
10 966g for animals inoculated with the mix of three strains. For the sham inoculated pigs,
11 their ADG were 953 and 908g in Pens b and f respectively. These values appeared similar
12 to those of the negative control animals in Unit 4 (ADG of 921g).

13

14 **3.2 Bacteriological results and kinetics of excretion**

15 Before inoculation, no *Campylobacter* was detected in the environment (wall and floor
16 swabs, feed and air samples) and in the pig faeces. The number of CFU per gram of
17 faeces excreted until 80 days p.i. (post-inoculation) are presented in Figure 2. Two days
18 after inoculation, all the animals infected by the porcine *C. coli* strain (Unit 1, Pen a)
19 excreted from 10^3 to 10^7 CFU/g of faeces for most pigs. The excretion was continuous
20 during the entire test with a slight decrease at the end of the fattening period
21 (approximately 10^4 CFU/g of faeces). No *Campylobacter* was found in the faeces of one
22 animal at 35 days p.i..

23

24 Animals inoculated with poultry *C. coli* (Pen c) excreted at the beginning of the trial a
25 lower quantity of *Campylobacter* compared to the pigs inoculated with porcine *C. coli*

1 (Pen a) (4×10^3 , 4.7×10^4 and 3×10^5 CFU/g of faeces versus 1×10^6 , 3.7×10^6 and 5.6×10^5 CFU/g
 2 of faeces respectively). Moreover, at the last sampling time (80 days post-inoculation),
 3 the level of *Campylobacter* in the faeces slightly decreased. At the end of the trial,
 4 *Campylobacter* was detected only in faeces of one pig and in caecal content for another
 5 pig.

6
 7 Two days after the inoculation, only one animal inoculated with *C. jejuni* (Pen d)
 8 excreted *Campylobacter* (3×10^3 CFU/g of faeces). After eight days, the three animals
 9 excreted respectively 1×10^2 , 9×10^3 and 6×10^3 CFU/g of faeces. The level of *Campylobacter*
 10 in the faeces rapidly decreased and *Campylobacter* was not found from the 21st day for
 11 one animal, from the 35th day for the second and from the 49th day for the third. Re-
 12 excretion of *Campylobacter* was observed for one pig at 56 days post-inoculation.

13
 14 For the animals inoculated with a mix of the three strains (Pen e), the level of
 15 *Campylobacter* excreted ranged from 10^3 to 10^7 CFU/g of faeces. This excretion was
 16 observed at all sampling times and the average level of *Campylobacter* in the faeces was
 17 around 10^5 CFU/g at 80 days post-inoculation. At 49 days after the inoculation, one pig
 18 excreted 2×10^7 CFU/g. This increase was associated with diarrhoea.

19
 20 At the different times of sampling, no *Campylobacter* was ever found in the faeces of the
 21 negative control pigs. On the contrary, pigs in the adjacent pens (sham inoculated pigs)
 22 shedded *Campylobacter* 21 days after inoculation. Their excretion, quantified from the
 23 49th day after inoculation, was continuous and between 10^3 and 10^6 CFU/g of faeces,
 24 similar to those of the inoculated pigs housed in the same unit.

25

1 **3.3 Species identification and distinction of porcine and poultry *C. coli* by PFGE**

2 All the isolates collected from faecal samples and caecal contents at the end of the trial
3 were analysed by PCR. For Unit 1, all of them were identified as *C. coli*. PCR confirmed
4 that isolates excreted by pigs in Pens c and d were always *C. coli* and *C. jejuni*
5 respectively, suggesting the absence of *Campylobacter* transmission between Pens c and
6 d. All the isolates from the pigs inoculated with the three strains and from the sham
7 inoculated pigs (Pens e and f) were identified as *C. coli*. In unit 3, PFGE was used to
8 identify the animal origin of the *C. coli* isolates of the pigs inoculated with the three
9 strains and the sham inoculated pigs (Figure 3). Porcine and poultry *C. coli* isolates
10 excreted by each pig are presented in Table I. Of the 20 isolates studied per pig, one pig
11 (numbered 1 in Pen e) was only excreted *C. coli* of porcine origin. The others, inoculated
12 pigs and sham inoculated pigs, excreted *C. coli* of porcine origin and *C. coli* of poultry
13 origin.

1 4 DISCUSSION

2 This trial assessed quantitatively the *Campylobacter* excretion by the pigs of three strains
 3 from different origins during 80 days post-inoculation. It also demonstrated the
 4 transmission of *Campylobacter* from the inoculated pigs to the neighbouring animals
 5 housed in adjacent pens. To our knowledge, this trial is the first reported experimental
 6 infection of weaners with *Campylobacter*. These weaned animals, with established
 7 intestinal flora, had never been in contact with *Campylobacter* before the inoculation.
 8 Moreover, the control pigs remained negative throughout the experimentation. This
 9 allows to exclude a possible contamination by exogenous factors entering the barn such
 10 as food, water, uncontrolled air flow or handlers. The presence of *Campylobacter* in the
 11 digestive tract of piglets did not lead to health disorder occurrence and/or a decrease in
 12 growth performances.

13
 14 In the present study, two days after inoculation, the six animals infected with *C. coli* of
 15 porcine origin (alone or in a mix) became positive and excreted *Campylobacter*. This
 16 finding was also observed for the neighbouring sham inoculated animals which excreted
 17 *Campylobacter* at the same level at the end of the trial. The levels of excretion were
 18 similar to those observed in the fattening pigs after natural infection (Weijtens et al.,
 19 1993; Harvey et al., 1999; Weijtens et al., 1999; Weijtens et al., 2000). These results did
 20 not seem to be influenced by the simultaneous inoculation of three *Campylobacter*
 21 strains, which lead to a higher infection dose (15×10^7 versus 5×10^7).

22
 23 The average colony count of *Campylobacter* in the faeces decreased slowly with the age
 24 of the animals. Weijtens et al. (1993) have shown that the percentage of carrier pigs
 25 tended to decrease during the fattening period (95% of positive samples at the beginning,

1 85% at the end of the fattening period with a smaller quantity of *Campylobacter* in the
2 faeces). A more rapid decrease in the excretion level was reported in Weijtens et al.
3 (1999) as well as in our study, compared to Weijtens et al. (1993). It could be due to the
4 fact that the two former studies were experiments with strict hygiene management
5 whereas the latter was a field study. Indeed, in our study, there was a combination of a
6 high level of cleaning with good living conditions for the pigs (housed in pens with a
7 restricted number of animals, in a quiet environment and fed *ad libitum*).
8
9 *Campylobacter* could not be detected in one animal at one time point whereas high counts
10 were observed in faeces from the same pig at previous and later sampling times. These
11 observations suggest an intermittent excretion of *Campylobacter*. Moreover, similar to
12 previous findings (Weijtens et al., 1999), variations in the number of *Campylobacter* in
13 the faeces between both animals and samples from a given animal were observed in our
14 trial. Lee et al. (1986) showed in a germfree adult mouse model that *C. jejuni* colonized
15 the intestinal tract *via* an association with the intestinal mucus layer on the surface
16 mucosa or in the intestinal crypts and did not adhere to the epithelial cells. In addition,
17 Hugdhal et al. (1988) and Takata et al. (1992) noticed the chemotactic behavior of *C.*
18 *jejuni* and its importance for the effective colonization of the intestinal tract, especially in
19 the mucus layer, where more chemoattractant might be present than in the gut space.
20 These results suggest an heterogeneous distribution of *Campylobacter* in the gut content
21 and consequently in samples taken from the rectal content. This could explain the
22 discrepancies between quantities of *Campylobacter* observed in successive samples.
23 Furthermore, the result with one pig exhibiting a negative faecal sample and a positive
24 caecal sample could favour this assumption.

25

1 In our trial, three strains of *Campylobacter* differing in species (*C. coli* and *C. jejuni*) and
 2 in origin (pig and poultry) were tested to evaluate a possible differential infection
 3 potential and the possible co-existence of different strains in pigs. Animals inoculated
 4 with the poultry *C. coli* strain excreted lower quantities of bacteria compared to the
 5 animals inoculated with the porcine *C. coli* strain and their excretion period was shorter.
 6 *Campylobacter* was not any more present in faeces of pigs inoculated with *C. jejuni*.
 7 Moreover, we could not evidence *C. jejuni* by PCR identification for (i) the animals
 8 inoculated with the mix of strains and (ii) the neighbouring ones. Our results are
 9 supported by reports from other European countries (Denmark, Portugal and Italy),
 10 showing low prevalences of *C. jejuni* in pigs (Cabrita et al., 1992; Nielsen et al., 1997;
 11 Pezzotti et al., 2003). Recently Jensen et al. (2006) have investigated the occurrence and
 12 species distribution of thermophilic *Campylobacter* in organic outdoor pigs in Denmark.
 13 They found that all the pigs excreted *Campylobacter*; *C. jejuni* was always detected to a
 14 lesser extent than *C. coli* and individual pigs were *C. jejuni* positive just once or in non-
 15 consecutive weeks. This indicates that the presence of *C. jejuni* in pigs may be sporadic
 16 and/or that the detection of low numbers of *C. jejuni* in pigs is difficult. Boes et al. (2005)
 17 observed that pigs were unfrequently infected by *C. jejuni* even in farms where *C. jejuni*
 18 infected cattle or poultry had been demonstrated. Some studies in the United States or in
 19 Canada have reported higher *C. jejuni* prevalences of 30 to 50% in slaughterhouse pigs
 20 and pork (Finlay et al., 1986; Harvey et al., 1999; Young et al., 2000). Different
 21 hypotheses can explain the discrepant *C. jejuni* finding in pigs among these studies: (i)
 22 the species identification method which may influence the result as demonstrated
 23 previously for the hippurate test (Englen et al., 2003; Waino et al., 2003) or (ii) the
 24 bacteriological method especially in the enrichment step (Manser and Dalziel, 1985;
 25 Richardson et al., 2001). Our results suggest that *C. coli*, especially the porcine strain, is

1 more likely to colonize the digestive tract in pigs compared to poultry *C. coli* or *C. jejuni*
 2 strains. These findings suggest a preferential association between some *Campylobacter*
 3 strains and some animal species. These associations between hosts and *Campylobacter*
 4 strains have been confirmed by several authors through genotyping techniques. Dingle *et al.*
 5 (2005) using a MLST technique observed that chickens and pigs located on a same farm
 6 were colonized with different STs indicating a host preference by some *C. coli* genotypes.
 7 Host-associated alleles by MLST of *C. coli* were also identified in the USA by Miller *et*
 8 *al.* (2006). Using the AFLP method, genetic separation between *C. coli* from poultry and
 9 *C. coli* from pigs were described by Hopkins *et al.* (2004) and by Siemer *et al.* (2005).

10

11 To summarize, the transmission of *Campylobacter* was demonstrated between pens and
 12 all the neighbouring animals became shedders with a level of excretion similar to
 13 inoculated pigs. This transmission could be demonstrated for the two *C. coli* strains.
 14 These results highlighted the possible between-pen transmission, given that pens may
 15 harbour perforated separations in commercial herds. Our experimental trial confirmed
 16 field results like the level of *Campylobacter* excretion, the intermittence in the excretion of
 17 the bacteria by pig. Finally, this trial illustrated the preferential association between
 18 *Campylobacter* species/strains and host animals species : *C. coli* seems to have a higher
 19 capacity to colonise the pig digestive tract.

20

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23

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Figure 1: Experimental design of the trial

Unit 1: Pen a, pen with three pigs inoculated with the porcine *C. coli* strain (◆) and **Pen b**, pen with three non-inoculated pigs (◇); **Unit 2: Pen c**, pen with three pigs inoculated with the poultry *C. coli* strain (▲) and **Pen d**, pen with three pigs inoculated with the poultry *C. jejuni* strain (●); **Unit 3: Pen e**, pen with three pigs inoculated with a mix of the three strains (■) and **pen f**, pen with three neighbouring non-inoculated pigs; **Unit 4: Pen g**, pen with the three negative control non-inoculated pigs.

Figure 2: Number of CFU of *Campylobacter* per gramme of faeces excreted by pigs during 80 days from inoculation.
Unit 1: **a**, pen with three pigs inoculated with the porcine *C. coli* strain; **b**, pen with three neighbouring non-inoculated pigs; **Unit 2:**
c, pen with three pigs inoculated with the poultry *C. coli* strain; **d**, pen with three pigs inoculated with the poultry *C. jejuni* strain;
Unit 3: **e**, pen with three pigs inoculated with a mix of the three strains; **f**, pen with three neighbouring non-inoculated pigs.

1 **Figure 3: PFGE profiles with *Sma*I enzyme from the 20 isolates of the pig**
2 **number 5 in Pen f (Table 1).**

3 Lanes 1, 12, 23 : lambda ladder 50-1000kb; Lanes 3, 4, 6, 9, 10 : porcine *C. coli*
4 isolates ; Lanes 2, 5, 7, 8, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 : poultry *C. coli*
5 isolates; Lane 24, reference profile for porcine *C. coli* strain, Lane 25 : reference
6 profile for poultry *C. jejuni* strain, Lane 26 : reference profile for poultry *C. coli*
7 strain.

Table 1: Number of isolates per origin among the 20 isolates collected at the end of the trial from pigs in unit 3. Three pigs (1, 2, 3) were inoculated with the three strains and three pigs (4, 5, 6) were housed in the adjacent pen.

Animals	Isolate species and origin		
	Porcine <i>C. coli</i>	Poultry <i>C. jejuni</i>	Poultry <i>C. coli</i>
Inoculated pigs (Pen e)			
1	20	0	0
2	17	0	3
3	13	0	7
Neighbouring Pigs (Pen f)			
4	13	0	7
5	5	0	15
6	6	0	14

4