

## Transmission of F4+ *E. coli* in groups of early weaned piglets

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### SUMMARY

The aim of this study was to estimate transmission parameters of enterotoxigenic F4+ *Escherichia coli* F4 (F4+ *E. coli*) in groups of early weaned piglets with F4-receptor-positive (F4R+) and F4-receptor-negative piglets (F4R–). Transmission of F4+ *E. coli* was quantified in four heterogeneous groups of F4R+ and F4R– piglets. Infectiousness was determined by the number of F4+ *E. coli*/g faeces shed during 8 days. Transmission parameters were estimated using generalized linear models assuming a stochastic SIR model. F4R+ piglets were found to be more susceptible than F4R– piglets, but F4R+ and F4R– piglets were not different in infectiousness. The reproduction ratios for homogeneous F4R+ and F4R– populations were estimated as 6·37 (95% CI 1·89–21·48) and 0·02 (95% CI 0·00–1·13) respectively. The implication of these results is that in order to prevent major outbreaks, the fraction of F4R+ piglets should be small (approximately 10% or less). Therefore, selective breeding programmes could contribute to reducing F4+ *E. coli*-related diarrhoea and transmission.

### INTRODUCTION

In 2001, an evaluation of the importance of several diseases for the Dutch pig farming was undertaken, and it was reported that post-weaning diarrhoea (PWD) is one of the diseases which causes substantial damage [1]. PWD is associated with the colonization and mass proliferation of enterotoxigenic *Escherichia coli* strains in the small intestine of newly weaned piglets [2].

At the moment there are no effective vaccines available [3, 4] and it is feared that the present more restrictive use of antimicrobial growth promoters in the European Union and a complete ban of these growth promoters by 2006 will lead to an increase in

diarrhoea incidence after weaning [5]. Therefore, other measures have to be investigated that either prevent clinical symptoms by directly interfering on the individual level or by preventing transmission of the bacterium between piglets.

Adherence of *E. coli* to the small intestine is an important step in the colonization and massive replication of *E. coli* [6]. Several adherence factors have been described and enterotoxigenic *E. coli* strains with adhesin F4 (or K88) have been reported as the main agents of PWD in several countries [7–9].

It was shown that F4+ *E. coli* do not adhere to the small intestine of all piglets, some piglets do not express the adherence site, F4 receptor (F4R) [10]. The adherence strategy of F4+ *E. coli* and the differences in expression of the receptor offers opportunities to prevent F4+ *E. coli* infections for example by F4-based oral vaccines [3, 4] and genetic selection for F4R– piglets [11]. Selection for F4R– piglets could

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be a good option to interfere in both F4+ *E. coli* diarrhoea and transmission. To what extent this measure will affect transmission can be quantified in transmission experiments.

One-to-one transmission experiments, in which one infectious pig is housed with one susceptible pig, have the advantage that, within a pair of piglets it is clear who infected whom. Results of a one-to-one transmission experiment with F4+ *E. coli* showed that F4R- piglets were less susceptible for F4+ *E. coli* infection than F4R+ piglets [12]. Results of this experiment were inconclusive on whether F4R+ and F4R- piglets differed in infectiousness, as none of the inoculated F4R- piglets was infectious, probably due to a low infection pressure. The implications of these findings are that the fraction of F4R+ piglets in a population will have influence on F4+ *E. coli* transmission.

To further investigate the effect of the fraction of F4R+ piglets in a population we estimated transmission parameters in groups of early weaned piglets. The group experiments provide new data in addition to the data from the one-to-one transmission experiment. The new data is important for a more accurate estimation of the transmission parameters and for insight in the interaction of piglets with different shedding patterns and different receptor status.

## MATERIALS AND METHODS

### Experimental design

Forty-eight castrated male piglets, age 3–4 weeks, were brought from a commercial farm in The Netherlands to the Animal Sciences Group at the day of weaning (day 0).

The piglets were obtained from 14 litters of 1–5 parity sows. Upon arrival piglets were weighed (weights 4.3–11.3 kg) and rectal swabs were taken and were checked for haemolytic *E. coli*. Piglets were housed in four groups of 12 piglets, one group per stable. They were assigned randomly to the groups with the restriction that littermates were not housed in the same group and that the weights of the piglets were equally distributed over the groups. All pens consisted of wire-mesh partitions that were placed on grid floors, with one piglet per 0.45 m<sup>2</sup> floor surface. The mean temperature of the stables was 25 °C with a 16-h light/8-h dark cycle. During the experiment the pens were not cleaned to ensure a maximum infectivity in the pen.

On days 0 and 1, piglets were fasted with water available *ad libitum*, from day 2 piglets were fed *ad libitum* with standard commercial piglet feed containing 18.9% crude protein (Hope Farms bv, Woerden, The Netherlands). This diet did not contain any antibiotics.

On day 4, all piglets were inoculated orally with 2 ml of a rotavirus strain RV277 suspension. On day 5, six randomly chosen piglets per group were orally inoculated with 5 ml of 10<sup>9</sup> c.f.u./ml F4+ *E. coli* suspension [Animal Sciences Group (ASG), Lelystad, The Netherlands]. Rectal faecal samples were collected daily of both inoculated and contact piglets and the number of F4+ *E. coli*/g faeces and the percentage dry matter of these samples was determined. Faeces were also examined and a 4-point scoring scale (0=normal, 1=unformed or loose consistency, 2=pasty diarrhoea and 3=liquid) was used by the animal caretakers to describe the consistency. During the experiment the health of the piglets was recorded daily. On day 19 piglets were weighed, euthanized, bled and necropsied. A 5–10 cm jejunal sample was taken for determination of the F4R status by brush border adhesion assay (BBA) [10].

For the record, we point out that biopsies were taken of the small intestine of the piglets in group 4 on day 1 for future research possibilities. Before the biopsies were taken, piglets were sedated with azaperone (2.0 mg/kg; Stresnil<sup>®</sup>, Janssen-Cilag, Tilburg, The Netherlands) and were anaesthetized using 3% sevoflurane (Sevorance<sup>®</sup>, Abbott, Zwolle, The Netherlands), (1 l O<sub>2</sub> and 0.8 l N<sub>2</sub>O), no medication was given. As far as we could ascertain, this did not affect the health of the individual piglets and could not have interfered with our study. The local Ethics Committee for Animal Experiments approved the experimental protocols.

### Inoculation

Rotavirus strain RV277 is maintained at the laboratory facilities of the Animal Sciences Group and was originally isolated from piglets with rotaviral neonatal diarrhoea. The average virus concentration, determined by negative stain electron microscopy, was 1.0 × 10<sup>6</sup> particles/ml. Inoculation with rotavirus preceding F4+ *E. coli* inoculation was chosen, because in our experience, rotavirus is a predisposing factor for F4+ *E. coli* infections and is often found as a co-infection in the field.

*E. coli* serotype O149:K91:F4ac (LT+, STb+), strain CVI-1000 (ASG, Lelystad) [13], was isolated from a pig farm with PWD. This strain was found to be resistant to a combination of streptomycin, tetracycline and vancomycin, which was therefore added to selective His-agar to prevent overgrowth by other bacteria (see also 'Determination of c.f.u. F4+ *E. coli/g* faeces' section below). As a negative control in the BBA, *E. coli* strain CVI-1084 (ASG, Lelystad) was used. This is also an O149:K91 (LT+, STa+) strain, but without fimbrial expression of F4. The strains were grown overnight in brain-heart infusion broth (Difco Laboratories, Detroit, MI, USA) at 37 °C, pelleted by centrifugation, resuspended in PBS at pH 7.2 (Biotrading, Mijdrecht, The Netherlands), to an optical density value of 1.050 at 600 nm which corresponds to a suspension of 10<sup>9</sup> c.f.u./ml.

#### Faecal dry matter and faecal scores

Faeces (0.8–4.3 g) were weighed into aluminium trays. Samples were desiccated for 22 h in an incubator at 80 °C, and weighed again to determine lost water.

#### Determination of c.f.u. F4+ *E. coli/g* faeces

Of ten-fold dilutions of faeces homogenized in saline (Biotrading), 100 µl was plated on selective His-agar plates containing 5% sheep blood, 50 µg/ml streptomycin, 25 µg/ml tetracycline and 50 µg/ml vancomycin (Biotrading). Haemolytic colonies of F4+ *E. coli* were counted with a lower limit of 100 c.f.u. F4+ *E. coli/g* faeces. In cases of uncertainty on the colony morphology, identity was confirmed by slide agglutination to establish the *E. coli* OK type (ASG, Lelystad).

#### Determination of F4R status

Receptor status of the duodenal biopsies taken from the piglets in group 4 at day 1 were determined as described below.

At necropsy, 5–10 cm of jejunal mucosa was scraped off of all piglets including the piglets in group 4. Epithelial brush borders were prepared to determine the F4R status of the piglets modified after Sellwood et al. [10]. Mucosal scrapings were placed in PBS containing 0.005 M EDTA (Merck, Germany) at 4 °C. Tissue was disrupted and dispersed by Ultrathorax, followed by filtration through a 100 µm mesh gauze. This filtrate was centrifuged for 10 min at

500 g to collect the cells. Cells were resuspended in PBS containing 0.05% D(+) mannose (Merck) and a CVI-1000 suspension of 0.25 ml containing 10<sup>9</sup> bacteria/ml PBS was added to 0.25 ml of the cell suspension. A second 0.25 ml cell suspension with a 0.25 ml CVI-1084 (F4–) suspension (10<sup>9</sup> bacteria/ml PBS) added, served as a negative control. The samples were gently mixed at room temperature for 45 min. A small aliquot was put on a slide under a coverslip, and bacterial adherence was determined by phase contrast microscopy (magnification 400×). Only cells with well-defined brush borders were studied. Animals with no or just 1–2 bacteria per brush border were considered F4R–; samples exceeding this were judged F4R+. In case of ambiguity, bacterial adherence of a new sample of the cell suspension with bacteria was determined.

#### Determination of shedding type

Piglets were classified as high and low shedders according to their F4+ *E. coli* shedding patterns of days 1–8 after inoculation. In an unpublished study by Geenen et al., a classification rule was determined by principal components analysis (PCA) to distinguish between high and low shedders. High shedding is associated with piglets being infectious [12]. All piglets of which the sum:  $\sum \text{coefficient } \ln \text{cfu}_k * (\ln \text{cfu}_k - \mu \ln \text{cfu}_k)$ , with  $k = \text{day } 1, 2, \dots, 8$  is smaller than 1.96 are high shedders.  $\ln \text{cfu}_k$  are the log-transformed numbers of (F4+ *E. coli/g* + 1) found in the faecal samples of days 1–8 after inoculation in the current study. For the contact piglets, day 1 was defined as the first day an F4+ *E. coli*-positive sample was found. For missing values the mean of the two surrounding values was filled in. The values of coefficient  $\ln \text{cfu}_k$  (the coefficients of the first eigenfunction resulting from PCA) and  $\mu \ln \text{cfu}_k$  [the mean of the log-transformed numbers of (F4+ *E. coli/g* + 1) in the population] were obtained from Geenen et al. (unpublished observations) and are given in Table 1.

#### Determination of diarrhoea and weight gain

For each set of faecal samples with a particular score (0–3), the mean percentage dry matter and 95% confidence intervals (CI) was calculated. Only piglets with one or more samples with a percentage dry matter below the upper limit score of 3 were considered to have severe diarrhoea. The association between absence or presence of severe diarrhoea and F4R

Table 1. *The coefficients of the first eigenfunction resulting from principal components analysis (coefficient ln cfu<sub>k</sub>) and the population mean (μ ln cfu<sub>k</sub>) of log-transformed F4+ E. coli/g data (days 1–8 after inoculation, n=69) obtained from Geenen et al. (unpublished observations), which were needed for the classification of high- and low-shedding piglets*

Day	Coefficient ln cfu <sub>k</sub> *	μ ln cfu <sub>k</sub> †
1	-0.17920	7.031
2	-0.34811	7.212
3	-0.39279	6.634
4	-0.44959	6.664
5	-0.43543	5.844
6	-0.38253	4.757
7	-0.33518	3.827
8	-0.20500	2.57

\* Coefficients of the first eigenfunction resulting from principal components analysis.

† Population mean of the log-transformed number of F4+ E. coli/g faeces.

status and between absence or presence of severe diarrhoea and classification as high and low shedders was studied using Fisher’s exact test for association.

Weight gain of the piglets was calculated as the mean weight over 19 days (g/day). It was tested whether F4R+ piglets or piglets with severe diarrhoea had a lower weight gain using the Mann–Whitney U test. Fisher’s exact test and Mann–Whitney U test were performed with GenStat [14].

**Determination of transmission parameters**

*Infection rate parameter*

For the calculations of the transmission parameters we assumed a stochastic SIR model [15]. In this model individuals can either be susceptible (S), infectious (I) or recovered and immune (R). New infections are assumed to occur at the rate (β·S·I)/N, where β is the infection rate parameter and N the total number of individuals.

The probability of one susceptible animal to become infected (a case) within an interval Δt is 1 - e<sup>-β·Δt·(I/N)</sup>. The number of cases (C) in a period Δt follows a binomial distribution with parameter 1 - e<sup>-β·Δt·(I/N)</sup> and index S, the number of susceptible individuals at the start of the period. Thus, the relation between the expected number of cases per unit of time E(C) and I, N, S and β is E(C)=S·(1 - e<sup>-β·Δt·(I/N)</sup>).

As F4R+ and F4R- piglets are thought to differ in susceptibility and infectivity, we distinguished between S<sub>p</sub> and S<sub>n</sub>, I<sub>p</sub> and I<sub>n</sub>, R<sub>p</sub> and R<sub>n</sub> individuals in which the subscript p stands for F4R positive and subscript n for F4R negative. This resulted in four different infection rate parameters; β<sub>pp</sub>, β<sub>pn</sub>, β<sub>np</sub> and β<sub>nn</sub>. Here the first character in the subscript is the F4R status of the contact piglet and the second is the F4R status of the infectious piglet (p=positive, n=negative). Thus, for F4R+ cases it applies that:

$$E(C_p) = S_p \cdot (1 - \exp\{-\beta_{pp} \cdot I_p + \beta_{pn} \cdot I_n\} \Delta t / N),$$

and for F4R- :

$$E(C_n) = S_n \cdot (1 - \exp\{-\beta_{np} \cdot I_p + \beta_{nn} \cdot I_n\} \Delta t / N).$$

From the experiment it is known between which subsequent samplings the contact piglets start excreting F4+ E. coli. It was assumed that a contact infection occurs one day before the contact piglets were found to start excreting F4+ E. coli.

Furthermore, it was assumed that in order to become a case, a piglet sheds a sufficient amount of F4+ E. coli to be infectious. ‘High shedders’ (see also ‘Determination of shedding type’ section above) were assumed to be infectious as this was found to be highly associated with the occurrence of cases in a one-to-one transmission experiment [12]. Since S, I, N and C are measured daily from the transmission experiment, we may drop Δt, being the unit of time, from the equation above, without loss of generality. With these measurements, the four βs were estimated using a generalized linear model (GLM) [16] with a complementary log-log link function and Log(I<sub>p</sub> + I<sub>n</sub>/N) as offset variable [17]. For F4R+ cases it applies that:

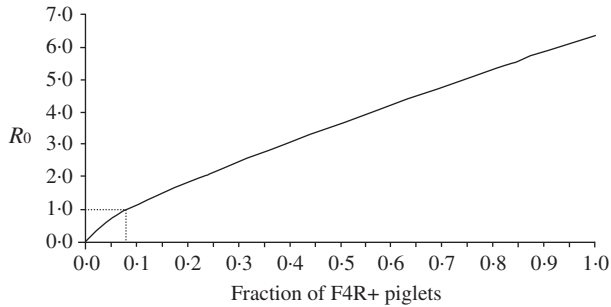
$$\begin{aligned} \text{Log}\left(-\text{Log}\left(1 - \frac{E(C_p)}{S_p}\right)\right) &= \text{Log } \beta + \text{Log}\left(\frac{I_p + I_n}{N}\right) \\ &= a + b \cdot q + \text{Log}\left(\frac{I_p + I_n}{N}\right) \end{aligned}$$

and for F4R- cases:

$$\begin{aligned} \text{Log}\left(-\text{Log}\left(1 - \frac{E(C_n)}{S_n}\right)\right) &= \text{Log } \beta + \text{Log}\left(\frac{I_p + I_n}{N}\right) \\ &= c + d \cdot q + \text{Log}\left(\frac{I_p + I_n}{N}\right), \end{aligned}$$

with

$$q = \frac{I_p}{I_p + I_n}$$



**Fig.**  $R_0$  plotted as a function of the fraction of F4R+ piglets in a population, with transmission rate parameters  $\beta_{pn}=1.42$ ,  $\beta_{pp}=0.77$ ,  $\beta_{np}=0.05$ ,  $\beta_{nn}=0.00$  and the average infectious periods  $T_n=10.8$  and  $T_p=8.3$ .

and

$$\beta_{pn}=e^a, \beta_{pp}=e^{a+b}, \beta_{np}=e^{c+d}, \beta_{nn}=e^c.$$

GLMs were performed with GenStat [14].

#### Reproduction ratio ( $R_0$ )

An insightful way to express the transmission parameters is by the  $R_0$  which is defined as the average number of secondary infections that one typical infectious individual will cause during its entire infectious period in a population of susceptible individuals only [18].  $R_0$  for the model described above is  $R_0=\beta \cdot T$ , where  $T$  is the average infectious period.  $T$  is estimated as the number of days from the first until the last F4+ *E. coli*-positive sample, which is consistent with the infectious period for the estimation of the infection rate parameters.  $R_0$  for heterogeneous populations (that is populations with F4R+ and F4R-) was calculated depending on the fraction of F4R+ piglets ( $f$ ) in the population.  $R_0$  is the dominant eigenvalue of matrix  $\mathbf{K}$ :

$$\mathbf{K} = \begin{pmatrix} f \cdot \beta_{pp} \cdot T_p & f \cdot \beta_{pn} \cdot T_n \\ (1-f) \cdot \beta_{np} \cdot T_p & (1-f) \cdot \beta_{nn} \cdot T_n \end{pmatrix}.$$

From this it follows that  $R_0(f) = \frac{1}{2}(k_{11} + k_{22} + \sqrt{(k_{11} + k_{22})^2 - 4 \cdot (k_{11}k_{22} - k_{12}k_{21})})$  [19] and  $R_0(f)$  for the estimated  $\beta$ s and  $T$ s is shown in the Figure.

## RESULTS

### Mortality and F4R status

Two piglets had to be killed for ethical reasons and were removed from the groups before F4+ *E. coli* inoculation. Therefore, groups 2 and 4 consisted of five inoculated and six contact piglets. During the

experiment, one piglet (6760) with symptoms of severe PWD was found dead at day 3 after F4+ *E. coli* inoculation. Receptor status of this piglet could not be determined, but because of the severity of the diarrhoea and the high number of F4+ *E. coli* shed, it was assumed to be an F4R+ piglet. Of the other 45 piglets, 21 were F4R+ and 24 were F4R-. Distribution of F4R+ and F4R- over the groups is shown in Table 2.

### Bacteriological examination and determination of shedding type

No haemolytic *E. coli* were found on the rectal swabs upon arrival. Table 2 shows the results of the determination of the number of F4+ *E. coli*/g faeces (the results shown are in c.f.u./g, not log-transformed) in the faecal samples collected after inoculation. Only six out of 46 piglets had no F4+ *E. coli*-positive samples during the experiment. They were all F4R-, five piglets were housed in group 3.

Shedding status of piglet 6760 could not be determined since only samples of days 1 and 2 p.i. were obtained. For the estimation of the transmission parameters it was assumed to be a high shedder and thus, infectious, considering the high number of F4+ *E. coli* in the first faecal samples. Of the 40 piglets with at least one F4+ *E. coli*-positive sample or more, 26 were determined to be high shedders and 14 low shedders. Association of receptor status and shedding type of the inoculated piglets is highly significant ( $P < 0.01$ , Fisher's exact test).

### Clinical parameters

#### Clinical scores

Of the 644 faecal samples collected, 361 were scored normal, 187 with unformed or loose consistency, 57 with pasty diarrhoea and 39 with liquid diarrhoea. The mean percentage dry matter of the samples with scores 0-3 and the 95% CIs are shown in Table 3. Nine piglets (3 inoculated and 6 contact piglets) had one or more samples with a percentage dry matter  $\leq 10.6\%$ , the upper limit of the 95% CI of score 3, and were classified as severely diarrhoeic. All nine piglets were F4R+ and high shedders. Both receptor and shedding status were highly associated with the occurrence of liquid stools ( $P < 0.01$ , Fisher's exact test). Only four out of 39 samples of liquid stools were found negative for F4+ *E. coli* on the

Table 2. Number of F4+ *E. coli*/g faeces (c.f.u./g, not log-transformed), receptor status and shedding type of piglets in groups 1–4

Group	Receptor	Pig no.*	1 d	2 d	3 d	4 d	5 d	6 d	7 d	8 d	9 d	10 d	11 d	12 d	13 d	14 d	Shedding type
1	–	6741i†						$1.0 \times 10^3$	$1.0 \times 10^4$	$2.0 \times 10^3$	$3.2 \times 10^4$	$7.0 \times 10^4$	$1.0 \times 10^2$				High
1	–	6742i	$1.8 \times 10^4$	$6.0 \times 10^2$				$2.0 \times 10^2$	$5.0 \times 10^3$	$1.0 \times 10^3$	$2.0 \times 10^4$	$1.6 \times 10^4$		$1.0 \times 10^4$			High
1	+	6744i	$3.1 \times 10^8$	$1.7 \times 10^8$	$4.43 \times 10^8$	$1.6 \times 10^8$	$2.7 \times 10^6$	$1.6 \times 10^4$								$8.0 \times 10^2$	High
1	+	6745i	$4.9 \times 10^6$	$6.1 \times 10^6$	$5.52 \times 10^8$	$1.2 \times 10^8$	$1.3 \times 10^8$	$3.6 \times 10^7$	$5.6 \times 10^6$	$7.8 \times 10^5$	$3.9 \times 10^3$						High
1	+	6750i	$3.0 \times 10^4$	$1.2 \times 10^3$		$5.0 \times 10^2$		$3.8 \times 10^3$	$4.2 \times 10^6$								High
1	–	6752i								$3.7 \times 10^3$	$1.1 \times 10^4$						Low
1	+	6743c			$5.54 \times 10^4$	$3.5 \times 10^6$	$2.6 \times 10^7$	$2.4 \times 10^7$	$2.3 \times 10^6$	$2.5 \times 10^6$	$1.5 \times 10^5$	$5.0 \times 10^3$	$4.6 \times 10^3$	$1.7 \times 10^3$			High
1	–	6746c			$1.18 \times 10^3$				$6.2 \times 10^2$	$2.0 \times 10^2$	$1.8 \times 10^3$		$3.0 \times 10^2$				Low
1	+	6747c				$7.7 \times 10^4$	$4.0 \times 10^4$	$1.9 \times 10^5$	$1.8 \times 10^5$	$1.6 \times 10^3$	$1.7 \times 10^5$						High
1	+	6748c		$1.5 \times 10^4$	$9.20 \times 10^6$	$6.3 \times 10^7$	$4.0 \times 10^7$	$1.5 \times 10^8$	$3.6 \times 10^8$	$4.2 \times 10^8$	$1.5 \times 10^8$	$4.6 \times 10^4$	$8.0 \times 10^2$	$1.0 \times 10^3$	$4.0 \times 10^3$		High
1	+	6749c	$4.6 \times 10^5$		$3.80 \times 10^4$	$1.6 \times 10^5$	$8.7 \times 10^6$	$7.5 \times 10^7$	$2.2 \times 10^8$	$9.1 \times 10^8$	$3.6 \times 10^7$	$1.1 \times 10^7$	$7.2 \times 10^6$	$7.8 \times 10^5$	$1.2 \times 10^5$		High
1	+	6751c		$1.0 \times 10^4$	$2.00 \times 10^5$	$6.8 \times 10^6$	$1.5 \times 10^8$	$1.6 \times 10^6$	$5.2 \times 10^5$	$2.5 \times 10^4$	$2.8 \times 10^3$	$2.2 \times 10^2$	$7.4 \times 10^3$		mv		High
2	–	6753i	$1.2 \times 10^6$	$7.7 \times 10^3$		$3.2 \times 10^3$	$7.1 \times 10^3$	$9.0 \times 10^2$	$5.5 \times 10^3$	$2.4 \times 10^4$							High
2	+	6754i	$2.2 \times 10^6$	$1.5 \times 10^6$	$1.50 \times 10^6$	$6.5 \times 10^6$	$4.2 \times 10^6$	$1.4 \times 10^6$	$1.2 \times 10^4$	$6.0 \times 10^2$		$2.0 \times 10^3$					High
2	–	6758i	$2.3 \times 10^5$	$1.0 \times 10^3$		$1.0 \times 10^3$	$1.5 \times 10^3$	$6.0 \times 10^2$	$1.6 \times 10^2$	$2.6 \times 10^4$	$1.3 \times 10^3$	$1.0 \times 10^2$		$1.0 \times 10^2$			High
2	–	6759i						$1.5 \times 10^5$									Low
2	?	6760i	$3.9 \times 10^8$	$8.3 \times 10^8$	#	#	#	#	#	#	#	#	#	#	#	#	?
2	–	6755c		$2.0 \times 10^4$				$7.0 \times 10^3$	$2.4 \times 10^3$					$1.0 \times 10^3$			Low
2	+	6756c		$4.4 \times 10^5$	$2.60 \times 10^6$	$1.6 \times 10^7$	$1.0 \times 10^8$	$4.8 \times 10^7$	$1.8 \times 10^5$								High
2	+	6757c		$2.7 \times 10^8$	$6.30 \times 10^7$	$4.8 \times 10^7$	$2.2 \times 10^8$	$1.2 \times 10^8$	$2.8 \times 10^8$	$4.0 \times 10^9$	$3.8 \times 10^6$						High
2	+	6761c					$1.2 \times 10^5$	$1.3 \times 10^4$	$2.4 \times 10^5$	$4.5 \times 10^8$	$1.9 \times 10^6$	$9.0 \times 10^5$	$2.3 \times 10^5$	$7.0 \times 10^4$			High
2	–	6762c															Low
2	–	6764c							$2.6 \times 10^4$								Low
3	–	6765i															Low
3	–	6766i													$1.0 \times 10^2$		Low
3	–	6768i															Low
3	+	6769i	$2.5 \times 10^6$	$1.9 \times 10^6$	$5.00 \times 10^4$			$4.2 \times 10^5$	$5.7 \times 10^4$	$3.1 \times 10^4$	$8.0 \times 10^2$	$9.0 \times 10^3$	$1.5 \times 10^3$				High
3	–	6773i															Low
3	–	6774i	$3.0 \times 10^4$														Low
3	–	6767c															Low
3	+	6770c			$1.00 \times 10^4$			$9.2 \times 10^4$	$3.5 \times 10^5$	$1.4 \times 10^5$	$1.1 \times 10^7$	$2.9 \times 10^7$	$1.1 \times 10^5$	$4.9 \times 10^3$			High
3	+	6771c							$6.0 \times 10^2$				$1.0 \times 10^3$	$1.3 \times 10^3$			Low
3	–	6772c										$2.0 \times 10^4$	$3.8 \times 10^4$				Low
3	–	6775c				$1.0 \times 10^3$											Low
3	–	6776c															Low
4	–	6778i	$1.0 \times 10^3$	$1.0 \times 10^3$		$8.0 \times 10^2$	$1.5 \times 10^5$	$2.6 \times 10^3$		$1.0 \times 10^4$		$2.0 \times 10^2$	$4.1 \times 10^3$				High
4	–	6781i					$3.1 \times 10^4$		$2.4 \times 10^3$								Low
4	+	6785i	$4.5 \times 10^5$	$1.1 \times 10^4$	$5.00 \times 10^4$	$1.8 \times 10^5$	$1.9 \times 10^6$	$6.0 \times 10^2$	$1.0 \times 10^3$								High
4	+	6786i	$1.5 \times 10^6$	$2.6 \times 10^6$	$5.38 \times 10^7$	$5.0 \times 10^7$	$7.0 \times 10^7$	$1.7 \times 10^6$		$2.6 \times 10^4$	$2.9 \times 10^4$						High

4	+	6788i	$2.4 \times 10^8$	$7.0 \times 10^6$	$1.07 \times 10^8$	$2.8 \times 10^7$	$6.3 \times 10^7$	$4.0 \times 10^4$	$7.1 \times 10^4$		High
4	+	6777c	$3.7 \times 10^4$		$2.7 \times 10^3$	$2.0 \times 10^3$		$1.4 \times 10^4$			Low
4	-	6779c			$4.0 \times 10^2$	$1.4 \times 10^5$	$2.4 \times 10^3$				Low
4	+	6782c	$7.4 \times 10^6$	$1.06 \times 10^8$	$8.0 \times 10^8$	$5.5 \times 10^9$	$3.7 \times 10^8$	$5.3 \times 10^8$	$6.7 \times 10^8$	$9.0 \times 10^8$	$9.5 \times 10^8$
4	-	6783c			$2.35 \times 10^3$	$2.1 \times 10^4$	$4.7 \times 10^3$	$9.4 \times 10^2$	$8.9 \times 10^3$	$3.2 \times 10^5$	
4	+	6784c	$3.1 \times 10^3$	$2.17 \times 10^5$	$1.3 \times 10^6$	$1.7 \times 10^5$	$2.7 \times 10^6$	$4.8 \times 10^7$	$7.4 \times 10^6$	$1.0 \times 10^6$	
4	-	6787c				$1.0 \times 10^2$	$9.0 \times 10^2$				

\* A pig no. followed by i is an inoculated piglet, followed by c a contact piglet.  
 † For calculation of the transmission parameters this piglet was assumed to be contact-infected.  
 The shaded areas show the infectious periods of the individual piglets.  
 mv, Missing value.  
 #, Dead.

Table 3. Scores of the consistency of the faecal samples and the matching mean percentage faecal dry matter and 95% confidence interval (CI)

Score (consistency of faeces)	Mean percentage faecal dry matter (95% CI)
0, normal	23.3 (22.9–23.7)
1, unformed or loose consistency	19.5 (18.9–20.1)
2, pasty diarrhoea	15.3 (14.1–16.5)
3, fluid	8.7 (6.8–10.6)

same day. The time of onset of the diarrhoea (soon after inoculation), the high numbers of F4+ *E. coli* shed by most diarrhoeic piglets and the length of the excretion period indicate that F4+ *E. coli* successfully colonized the small intestine of most diarrhoeic piglets.

*Weight gain*

The mean weight gain of the F4R+ piglets was 230.3 g/day (s.d. = 106.3) and 231.1 g/day (s.d. = 83.9) for the F4R- piglets which is not significantly different,  $P=0.41$  (Mann-Whitney *U* test). For severely diarrhoeic piglets, the mean weight gain was 157.9 g/day (s.d. = 82.4) and for piglets with no or milder forms of diarrhoea the mean weight gain was 246.5 g/day (s.d. = 89.6) respectively, which is significantly different ( $P=0.02$ , Mann-Whitney *U* test).

**Transmission parameters**

Piglet 6741 (F4R-, group 1) was considered to be contact-infected instead of being infectious due to inoculation, as its first F4+ *E. coli*-positive sample was not found until day 6 after inoculation. Including piglet 6741 a total number of 13 contact infections were observed, two F4R- and 11 F4R+. The estimated  $\beta$ s and the matching 95% CIs are shown in Table 4.

When we compare the intervals of  $\beta_{pp}$  and  $\beta_{np}$  or the intervals of  $\beta_{pn}$  and  $\beta_{nn}$  we see that they do not overlap or only slightly overlap and, therefore, it can be concluded that F4R+ and F4R- piglets differ in susceptibility. The intervals of  $\beta_{pn}$  and  $\beta_{pp}$  or  $\beta_{np}$  and  $\beta_{nn}$  do overlap almost entirely, therefore we may not conclude that F4R+ and F4R- piglets also differ in infectiousness.

The periods in which we assumed piglets to be infectious are shown in Table 2. For the inoculated

Table 4. Estimates of the four transmission parameters and their 95% confidence intervals (CI)

Transmission parameter*	Estimate (95% CI)
$\beta_{pn}$	1.42 (0.06–34.81)
$\beta_{pp}$	0.77 (0.23–2.58)
$\beta_{np}$	0.05 (0.02–0.14)
$\beta_{nn}$	0.00 (0.00–0.10)

\* The first character in the subscript is the F4R status of the contact piglet and the second is the F4R status of the infectious piglet (p, positive; n, negative).

piglets, this resulted in  $T_p=8.3$  and  $T_n=10.8$  days, which were used for the calculation of the reproduction ratios.

$R_0$  calculated for homogeneous F4R+ piglet populations based on the four  $\beta$ s, was estimated as 6.37 (95% CI 1.89–21.48).  $R_0$  for homogeneous F4R– piglet populations was much smaller, 0.02 (95% CI 0.00–1.13).

In order to achieve  $R_0(f) < 1$ , the fraction of F4R+ piglets in the population must be lower than 0.08.

## DISCUSSION

In this study, transmission parameters were estimated in randomly mixed groups of F4R+ and F4R– piglets and it was investigated whether F4R+ and F4R– piglets differed in infectiousness and susceptibility. F4R+ piglets were more susceptible to F4+ *E. coli* infection than F4R– piglets, but were not more infectious. This confirms and completes the findings of a one-to-one transmission experiment [12] in which susceptibility was found to differ, but which was inconclusive on the infectiousness as none of the inoculated F4R– piglets became infectious. In the current study, six F4R– piglets did become infectious, four inoculated and two contact piglets. A higher infection pressure in groups compared to pairs and consequently more ingestion of bacteria from the environment in combination with non-specific binding of F4+ *E. coli* to the intestinal wall might have been the underlying cause. The results of the group transmission experiment has led to a more accurate estimation of the transmission parameters than the results of the one-to-one experiment. Moreover, transmission between group-housed piglets is more representative for the practical situation.

Whereas 51% of the F4+ *E. coli*-positive samples of infectious F4R+ reach numbers of  $10^6$  c.f.u./g or higher, only 2% (one sample) of the infectious F4R– piglets reaches this level. This indicates that the colonization of the infectious F4R– piglets was less effective than the colonization of the infectious F4R+ piglets, which is also reflected in the number of F4R+ and F4R– piglets with diarrhoea. Despite this difference in the level of shedding, infectiousness did not differ between F4R+ and F4R– piglets. Probably, the power of the experiment was too small as only four inoculated F4R– piglets were infectious.

In an earlier study, we developed an objective measure to distinguish between diarrhoeic and non-diarrhoeic piglets using PCA on the percentage dry matter data of F4+ *E. coli*-inoculated piglets (Geenen et al., unpublished observations). In this study we did not succeed as there was too much overlap in the data. Therefore, in the current study visual observation of fluid diarrhoea by the animal caretakers was linked to the percentage dry matter determined on the same samples. It was determined that piglets with one or more samples with a percentage dry matter  $\leq 10.6\%$  were severely diarrhoeic, which was valid for nine piglets. Using the same methods in a study with individually housed piglets, a threshold value of 12.6% dry matter was found. Using this threshold value in the current study, 11 piglets with severe diarrhoea would have been found, but the conclusions on the association with receptor and shedding status and conclusion on weight gain would have remained the same.

In this study, transmission was studied in groups of piglets of which the individual piglets differ in susceptibility that is mainly determined by the absence or presence of an adhesion site. From the estimated transmission parameters it was calculated that it is only possible to prevent major outbreaks when the fraction of F4R+ piglets in the population is lower than 0.08. This is a slightly lower but more accurate estimate than the fraction of 0.14 calculated from the data in the aforementioned one-to-one transmission experiment. The main conclusion remains that in order to reduce transmission sufficiently, the fraction of F4R+ piglets has to be small.

Although the intestinal receptor locus for F4+ *E. coli* has been mapped on porcine chromosome 13 [19], it has been shown that the expression of the receptor genes is not always complete and is influenced by epistatic inhibitor genes [20] making breeding programmes difficult. Moreover, selection



on F4R— piglets could have some disadvantages like the change in selection pressure on pathogenic *E. coli* strains or negative effects on production and welfare as the function of the genes involved are still unknown [21, 22]. Furthermore, different serological variants of F4 with different receptors exist. Other intervention measures could be more beneficial and more feasible than a programme for selective breeding and their effect on clinical symptoms and transmission should be quantified.

In the future, it is necessary that genetic research on the F4R genes should be executed not only to find a gene marker that makes it possible to test pigs on their receptor status in a sensitive and non-invasive way but also to give more insight into the possible side-effects of selection on F4R— pigs. Furthermore a cost-benefit analysis should be done and the long-term effects of the change in selection pressure on pathogenic *E. coli* should be monitored.

PWD is a multifactorial problem and post-weaning proliferation of enterotoxigenic *E. coli* is an important step in a complex process [23]. The problem will not be solved by removing the receptor gene(s) alone. In addition to genetic selection, conditions under which piglets are reared will have to be evaluated thoroughly.

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