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Classification of temporal profiles of F4+ *E. coli* shedding and faecal dry matter in experimental post-weaning diarrhoea of pigs

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SUMMARY

Enterotoxigenic F4+ *Escherichia coli* can colonize the intestine of pigs and cause diarrhoea. Our primary goal was to find a discriminant rule to discriminate between F4+ *E. coli* shedding profiles as this may reflect differences in the infectiousness of pigs. Our secondary goal was to find a discriminant rule to discriminate between diarrhoeic and non-diarrhoeic pigs. Repeated measurements (bacterial shedding and percentage dry matter of faeces) were taken of 74 weaned pigs that were infected experimentally with F4+ *E. coli*. These measurements were summarized into two new variables by means of a principal components analysis. Discriminant rules were derived based on these summary variables by fitting a mixture of normal distributions. Finally, the association between the classifications (as derived from the discriminant rules) and the occurrence in the pigs of the F4 receptor, an adhesion site for F4+ *E. coli*, was studied. We found that only the classification based on bacterial shedding allowed us to distinguish two significantly different groups of pigs (high and low shedders). Presence of the F4 receptor was associated strongly with pigs being high shedders.

INTRODUCTION

Enterotoxigenic *Escherichia coli* strains possessing fimbriae with adhesin F4 (F4+ *E. coli*) are an important cause of post-weaning diarrhoea (PWD) [1–3]. PWD is an infectious disease of newly weaned pigs and causes substantial weight loss and mortality.

Clinical symptoms after infection with F4+ *E. coli* have been studied well, but transmission of F4+ *E. coli* has not. Because an important characteristic of PWD is its contagiousness [4], the transmission characteristics of this infection should also be determined to support the development of proper control measures. However, before we were able to study transmission and diarrhoea among pigs, we needed to find a measure to discriminate between infectious and non-infectious pigs and a measure to discriminate between diarrhoeic and non-diarrhoeic pigs. Discrimination is far from straightforward since the colonization and replication of F4+ *E. coli* within the intestine [5] and clinical symptoms vary considerably [6–9]. The number of F4+ *E. coli* shed in the faeces as a function of time might be one of the measures of infectiousness of the pigs, which is of importance with respect to transmission. The degree of diarrhoea might also influence the spread of F4+ *E. coli* because it might produce aerosols (although oral uptake might be less).

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In vitro tests have shown that F4+ *E. coli* does not adhere to the brush borders of all pigs [10]. This difference is explained by the absence or presence of an F4 receptor (F4R) on the brush borders of the pigs, but the structure of F4R or the genes involved are not yet fully known. F4R promotes adherence of F4+ *E. coli* to the brush border of small-intestinal enterocytes and is important for the colonization and replication of F4+ *E. coli* in the small intestine, which is followed by production of enterotoxins (resulting in diarrhoea) [5]. In earlier studies, diarrhoea was found to be more prevalent in F4R+ pigs [7, 9, 11]. Pigs can be classified upon slaughter by *in vitro* adhesion assay [10] into F4R+ (adherent brush borders) and F4R− (non-adherent brush borders).

Three different antigenic types of F4 fimbriae have been described: F4ab, F4ac and F4ad. With regard to the attachment of these different antigenic types, six phenotypes of pigs have been found [12, 13].

F4+ *E. coli* was expected to replicate more within the intestine of F4R+ pigs than of F4R− pigs. This would lead to shedding of higher numbers of F4+ *E. coli* by F4R+ pigs. Consequently, F4R+ pigs are expected to be more susceptible to become infectious because entering the intestine would lead more often to colonization. F4R+ pigs could also be more infectious because they are expected to shed more bacteria and to have a longer excretion period. This is important for the course of transmission on the level of the population.

In this paper, our objective was to find a measure to discriminate between infectious and non-infectious pigs and a measure to discriminate between diarrhoeic and non-diarrhoeic pigs. Therefore, two discriminant rules were developed: one that discriminates between F4+ *E. coli* shedding profiles of individual pigs (regardless of their F4R status), and one that discriminates between the faecal dry matter profiles of individual pigs, because this is related to seriousness of diarrhoea and (possibly) to infectiousness.

Subsequently, we investigated the association between F4R status and both classifications as derived from the discriminant rules.

MATERIALS AND METHODS

Experimental design

We used data obtained from five experiments (Table 1) in which all piglets were inoculated with the same strain of F4+ *E. coli* [Animal Sciences Group (ASG), Lelystad, The Netherlands]. For our experiments, an F4ac strain was used and thus, in this paper F4+ *E. coli* refers to F4ac+ *E. coli* and F4R+ and F4R− refer to the F4Rac+ and F4Rac− pig phenotypes. For each piglet the number of colony-forming units of F4+ *E. coli*/g faeces at days 1–8 post-infection (p.i.), the percentage faecal dry matter (% DM) at days 1–8 p.i. and the F4R status was known. All experiments were carried out at the experimental facilities of the ASG.

All animals were purchased from a commercial piggery in The Netherlands and transported to the facilities on the day of weaning. All pigs were in good health, no haemolytic *E. coli* were found on rectal swabs taken upon arrival and none of the pigs had diarrhoea at weaning. The set-up of the experiments is summarized below.

Experiment 1. Ten male, castrated pigs were housed individually. At day 5 they were infected orally with 5 ml 10^8 c.f.u. F4+ *E. coli*/ml. Faeces were collected by colostomy pouches that were attached around the anus and the number of c.f.u. of F4+ *E. coli*/g faeces (denoted by CFU) and % DM were determined daily. At day 21, all pigs were anaesthetized, bled and necropsied and F4R status of the pigs was determined by brush-border adhesion assay (BBA) [10].

Experiment 2. Ten male, castrated pigs were housed individually. At day 4, pigs were infected orally with rotavirus (a predisposing factor for F4+ *E. coli* infection [14]) followed by oral infection with 5 ml 10^9 c.f.u. F4+ *E. coli*/ml on day 5. CFU and % DM were determined daily in faeces collected by colostomy pouches. F4R status was determined by BBA after euthanasia at day 19.

Experiments 3 and 4. Ten and 12 castrated pigs, respectively, were housed individually. Faecal samples were taken daily from all pigs directly from the rectum. Oral infection of rotavirus and F4+ *E. coli* was performed as described in experiment 2. CFU and % DM were determined on rectal faecal samples and F4R status was determined by BBA after euthanasia at day 19.

Experiment 5. Thirty-two pigs (17 male and 15 female) were housed in four groups of eight pigs. Pigs were assigned randomly to the groups with the restriction that littermates were not housed in the same group and that the weight and sex of the pigs were equally distributed over the groups. At day 4, all pigs

Table 1. *Summary of the five experiments in which 74 newly weaned pigs were inoculated with* E. coli *serotype O149:K91:F4ac (LT+, STb+), Animal Sciences Group, The Netherlands (April 2000–September 2001)*

Expt	No. of pigs	No. F4R+*	Housing	Inoculation†	Feed‡	Colostomy pouch	Weight at weaning Mean (S.D.)
1	10	3	Individual	(1)	(a)	Yes	6·4 (1·4)
2	10 (9)§	5 (4)§	Individual	(2)	(b)	Yes	6·9 (1·4)
3	10	3	Individual	(2)	(b)	5 yes, 5 no‖	6·8 (1·0)
4	12	4	Individual	(2)	(b)	6 yes, 6 no‖	8·1 (1·6)
5	32 (28)§	11 (10)§	4 groups of 8	(3)	(c)	No	6·9 (0·8)

* Determined at necropsy.
† (1) 5×10^8 c.f.u. F4+ *E. coli* on day 5 after weaning; (2) fasting on days 0 and 1, rotavirus on day 4 and 5×10^9 c.f.u. F4+ *E. coli* on day 5 after weaning; (3) fasting on days 0 and 1, colistin sulphate in drinking water days 0–4, rotavirus on day 4 and 5×10^9 c.f.u. F4+ *E. coli* on days 5 and 6 after weaning.
‡ (a) '5110p Superkorrel', 16·4% crude protein (Hendrikx, The Netherlands); (b) '2740 biggenbatterij 4 mm', 18·9% crude protein (Hopefarms bv, The Netherlands); (c) Weanling diet, 17·6% crude protein (Cehave, The Netherlands).
§ Values within parentheses are the number of pigs used in the statistical analysis when different from those given.
‖ For determination of F4+ *E. coli*/g faeces, rectal faecal samples were taken of all pigs.

were infected orally with rotavirus, followed by oral infection with 5 ml 10^9 c.f.u. F4+ *E. coli*/ml on day 5, which was repeated on day 6. CFU and % DM were determined on rectal faecal samples and F4R status was determined by BBA after euthanasia at day 14.

All experiments were performed with permission of the institutional local ethics committee for animal experiments. Differences in the design of the experiments led to variation in the day of euthanasia. This did not affected the F4R status of the pigs, because presence of the receptor in the brush-border fraction is independent of age [15].

Inoculation

E. coli serotype O149:K91:F4ac (LT+, STb+) was isolated from a pig in a farm with PWD and designated CVI-1000 (ASG, Lelystad) [16]. As a negative control in the BBA *E. coli* strain CVI-1084 (ASG, Lelystad) was used. This is also an O149:K91 strain (LT+, STa+) but without fimbrial expression of F4ac. The strains were grown overnight in brain heart infusion broth (Difco Laboratories, Detroit, MI, USA) and pelleted by centrifugation. The pellets were resuspended in PBS (pH 7·2) (Biotrading, Mijdrecht, The Netherlands) to an absorption value of 1·050 at 600 nm, which corresponds to a suspension of 10^9 c.f.u./ml.

Rotavirus strain RV277 was originally isolated from pigs with rotaviral neonatal diarrhoea and is maintained in the laboratory of the ASG. The average virus concentration (determined by negative-stain electron microscopy) was $1{\cdot}0 \times 10^6$ particles/ml. Inoculation with rotavirus in addition to inoculation with F4+ *E. coli* (except in experiment 1) was chosen because in our experience, rotavirus facilitates infection with F4+ *E. coli* and often is found as a co-infection in the field [14].

Analysis of faeces

Determination of % DM

Faeces (0·5–3·0 g) were weighed in aluminium trays. Samples were desiccated for 22 h in an incubator at 80 °C, and weighed again to determine lost water.

Determination of CFU

Ten-fold dilutions of faeces homogenized in saline (Biotrading) were done. Of each dilution, 10 μl was plated manually on selective His-agar plates containing 5% sheep blood, streptomycin (50 μg/ml), tetracycline (25 μg/ml) and vancomycin (50 μg/ml) (Biotrading). Plates with <200 haemolytic F4+ *E. coli* colonies were counted and the number of c.f.u./g faeces was calculated (lower limit 100 c.f.u./g faeces). In cases of uncertainty regarding colony morphology, identity was confirmed by slide agglutination to establish the *E. coli* OK type (ASG, Lelystad).

Determination of F4R status

Determination of F4R status by the BBA [10] was performed by laboratory technicians without prior knowledge of the performance of the pigs during the

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experiment, the bacterial counts or any information that possibly could be related to F4R status.

At necropsy, 5–10 cm of jejunal mucosa was scraped off and mucosal scrapings were placed in PBS containing 0·005 M EDTA (Merck, Darmstadt, Germany) at 4 °C. Tissue was disrupted and dispersed by Ultrathorax, followed by filtration through a 100-μm mesh gauze. The filtrate was centrifuged for 10 min at 500 ***g*** to harvest the cells. Cells were re-suspended in PBS containing 0·05% D(+) mannose (Merck). A CVI-1000 suspension (F4ac+) of 0·25 ml containing 10^9 bacteria/ml PBS was added to 0·25 ml of the cell suspension. The same was done with strain CVI-1084 (F4ac−), as a negative control. The samples were mixed gently 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 an average of 1–2 bacteria of strain CVI-1000 per brush border were considered F4R−; samples exceeding this were judged F4R+. In case of ambiguity, the test was repeated.

Statistical evaluation

Principal components analysis (PCA) was used in the analysis of the CFU and % DM measurements to summarize individual profiles in time. PCA is a statistical technique to derive a limited set of new (independent) variables (called principal components) that capture as much as possible of the variation in the original (dependent) variables. The principal components (pc's) are ordered with respect to decreasing variance. The percentage variance explained by, for example, the first two pc's (pc1 and pc2) is equal to

$$\frac{\text{variance of pc1} + \text{variance of pc2}}{\text{sum of the variances of the original variables}} \times 100\%.$$

All components together explain 100% of the data. A pc, e.g. pc1, is a linear combination of the original variables (y_1, y_2, …): $pc_1 = c_{11} * y_1 + c_{12} * y_2 + \cdots$, where the coefficients c_{11}, c_{12}, …(the loadings) are the elements of the eigenvector with the largest eigenvalue of the covariance matrix of the original variables.

When the original variables are associated with time points, the loadings can be used to derive 'eigenfunctions'. These eigenfunctions [17–19] reflect important characteristics to describe variation between individual profiles in time (e.g. increase over time or curvature). A profile (p) can be decomposed into a linear combination of the eigenfunctions ($f1$, $f2$, …): $p = pc_1 * f_1 + pc_2 * f_2 + \cdots$, where the coefficients are the values of the pc's of an individual piglet. The aim is to describe the variation between the profiles with a small number of eigenfunctions, i.e. to focus on the most important characteristics of the profiles. A pc indicates how strong such a characteristic is expressed in a profile of an individual piglet.

Missing values cannot be handled by PCA and were replaced by the average of the two neighbouring time points. An observation was considered to be a missing value when no faeces or insufficient faeces (<0·3 g) were collected to determine % DM and/or CFU. Data from animals that died during the experiments and data from animals with more than three missing values in CFU or % DM were considered unreliable and excluded from the analysis. In the event of observed absence of F4+ *E. coli* c.f.u./g faeces, CFU was set to 0. To obtain a more normal distribution in the statistical analysis, CFU data were log transformed; $\ln \text{CFU} = \ln(\text{CFU} + 1)$.

The analysis of the CFU and % DM profiles consisted of three steps.

Step 1. A separate PCA was performed for the CFU and % DM data. The first principal components of ln CFU and % DM, denoted by CFU PC_1 and % DM PC_1 respectively, were retained as a summary of the profiles. A biological interpretation of these pc's was inferred from the shape of the associated eigenfunctions (as visualized by plots of the loadings against time). These pc's will be used in subsequent steps for clustering and discrimination between pigs.

Step 2. Pigs were clustered into two groups on the basis of CFU PC_1 and % DM PC_1. CFU PC_1 and % DM PC_1 were assumed jointly to follow a mixture of two (bivariate) normal distributions. Each pig was assumed to follow one of the two normal distributions, under the assumption that there are two different shedding types and two different diarrhoea types of pigs. The proportions of pigs corresponding to the two distributions are the so-called mixture probabilities. These proportions, together with the means and variances of the underlying normal distributions, were estimated by maximum likelihood with the program EMMIX [20, 21]. Discrimination was based on the classical maximum-likelihood discriminant rule [22] that allocates an animal to the category where its observations have the greatest

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Table 2. *The median number of F4+* E. coli-*positive faecal samples per pig, the median number of F4+* E. coli *in the positive samples and the mean percentage faecal dry matter from all faecal samples of all pigs, the F4R+ and the F4R− pigs*

	All pigs ($n=536$)*	F4R+ pigs ($n=185$)*	F4R− pigs ($n=351$)*
Median (min – max) number of F4+ *E. coli* samples per pig	3 (0–8)	6 (0–8)	2 (0–8)
Median (min – max) number of F4+ *E. coli* in positive samples (c.f.u.)	7×10^4 (1×10^2–1×10^{10})	1×10^6 (1×10^2–1×10^{10})	7×10^3 (1×10^2–1×10^9)
Mean (± S.D.) percentage faecal dry matter	24·5 (±7·6)	21·7 (±9·3)	25·9 (±6·1)

* Number of faecal samples.

likelihood of appearing. To test whether the assumption of a mixture was tenable, the model consisting of a mixture of two normal distributions was compared with a model with a single common normal distribution by the likelihood ratio test (GenStat [23]).

Step 3. In this final step, the association between F4R status and the result of clustering of step 2 was studied in a 2×2 table, employing Fisher's exact test (GenStat [23]).

RESULTS

Of the 74 pigs, five pigs were excluded from analysis; four pigs had more than three missing values and one pig died of dehydration due to PWD. Of the remaining 69 pigs, 45 pigs were determined as F4R− and 24 F4R+. Nine pigs showed no F4+ *E. coli*-positive samples in the first 8 days after inoculation (two F4R+ and seven F4R−). In Table 2, the median number of F4+ *E. coli*-positive samples per pig, the median number of F4+ *E. coli* in the positive samples and the mean % DM from all faecal samples are shown for all pigs, the F4R+ and the F4R− pigs.

The first two pc's of ln CFU (ln CFU PC_1 and ln CFU PC_2) explained 76·2% and 9·3% of the variation in ln CFU, respectively (the associated eigenfunctions are shown in Fig. 1).

% DM PC_1 and % DM PC_2 explained 57·6% and 19·5% of the variation in % DM, respectively (their eigenfunctions are shown in Fig. 2). ln CFU PC_1 and % DM PC_1 explained the major part of the variation between the individual profiles; all other PCs explained only a relatively small amount of variation. The shape of the eigenfunctions associated with ln CFU PC_1 and % DM PC_1 (eigenfunction 1 in Figs 1 and 2) is basically constant, apart from a slight increase at the extremes. This suggests that major differences between the individual profiles of the animals are reflected by their overall level of ln CFU and their overall level of % DM. The eigenfunctions associated with ln CFU PC_2 and % DM PC_2

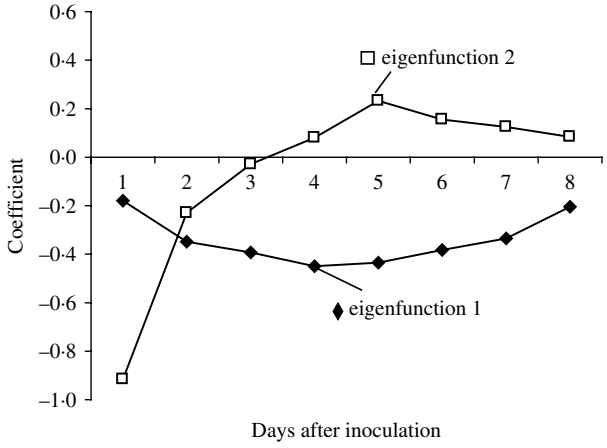

Fig. 1. Coefficients of the first and second eigenfunction of the log-transformed number of F4+ *E. coli*/g faeces data (ln CFU) plotted against time.

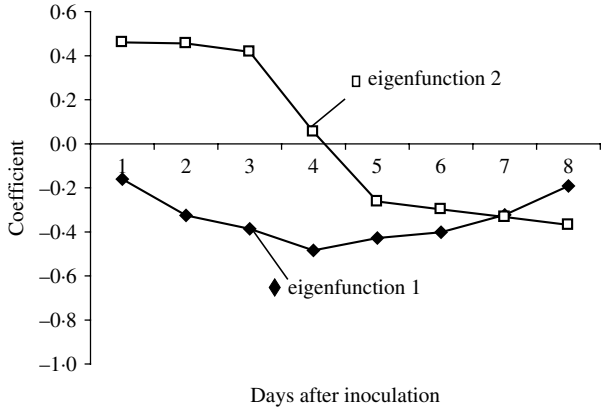

Fig. 2. Coefficients of the first and second eigenfunction of the percentage dry matter of faeces data (% DM) plotted against time.

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Table 3. *Classification results of the 69 pigs using the first or second principal component (PC_1 or PC_2) derived from Principal Component Analysis on the log-transformed number of F4+ E. coli/g faeces (ln CFU) or on the percentage dry matter of faeces (% DM)*

Summary variable	Proportion of pigs*		Mean†		Variance†		Likelihood ratio test *P* value‡	Cut-off value§
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2		
ln CFU PC_1	0·64	0·36	11·1	−18·6	0·00	16·2	0·00	1·96
ln CFU PC_2	0·52	0·48	−4·4	5·1	0·07	13·8	0·07	0·95
% DM PC_1	0·83	0·17	−5·7	27·3	0·06	113·0	0·06	11·65
% DM PC_2	0·94	0·06	−1·6	27·1	0·00	45·9	0·00	13·64

* The group with the highest proportion of pigs is referred to as group 1 and other as group 2.
† Means and variances of groups 1 and 2 estimated by maximum likelihood.
‡ Probability that pigs can be classified into two groups *vs*. one group.
§ Cut-off values between groups are calculated by the Maximum Likelihood Discriminant rule.

(eigenfunction 2 in Figs 1 and 2), show a contrast between the first and last days of the sampling period and largely represent a trend in time.

Classification results based on the PCs of ln CFU and % DM are summarized in Table 3. As can be seen from this table, only ln CFU PC_1 and % DM PC_2 could significantly discriminate between two types of pigs ($P<0·05$). Although, discrimination between pigs with high and low % DM summarized by % DM PC_1 was not significant ($P=0·06$), there was a strong indication that the pigs in group 2 (Table 3) suffered from diarrhoea. Classification based on % DM PC_2 was significant, but the amount of variation explained by % DM PC_2 was low (19·5%). Moreover, % DM PC_2 distinguished one group consisting of only four piglets with diarrhoea at days 5–8 p.i., and one group with all other 65 piglets. Therefore, % DM PC_2 was considered not to be a biological meaningful measure to discriminate between diarrhoeic and non-diarrhoeic piglets and was not evaluated further.

The major difference between the piglets was their overall level of bacteria shed at log scale, which was summarized by ln CFU PC_1. This means that it is possible to discriminate between pigs that shed high numbers of F4+ *E. coli*/g faeces at log scale (group 2, Table 3) and pigs that shed low numbers at log scale (group 1, Table 3). These two shedding types will, therefore, be referred to as high and low shedders.

Cut-off values for the different groups calculated by the maximum-likelihood discriminant rule are also shown in Table 3. For example, pigs with values of ln CFU PC_1 smaller than the boundary value 1·96 were classified as high shedders. Thus, the discriminant rule found to classify piglets as high shedder was: Σ_k coefficient$_k$ $*$ (ln $CFU_k - \mu$ ln CFU_k) $<1·96$ with $k=1$, 2, …, 8. ln CFU_k are the log-transformed numbers of F4+ *E. coli*/g+1 found in the faecal samples of individual piglets on days 1–8 after inoculation, μ ln CFU_k are the mean number of ln CFU shed at days 1–8 of all 69 piglets and coefficient 1_k are the coefficients (or loadings) of the first eigenfunction at days 1–8 (see Fig. 1).

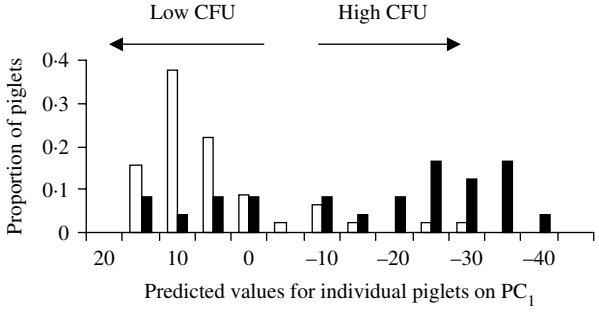

Fig. 3. Proportions of F4R+ pigs (■, $n=24$) and F4R− pigs (□, $n=45$) (all experiments combined) on the predicted values of the first principal component of the log-transformed number of F4+ *E. coli*/g faeces (ln CFU PC_1). Piglets with a predicted value of $<1·96$ were defined as high shedders.

In the last step of the analysis, it was investigated how the F4R+ and F4R− piglets were distributed over the shedding and diarrhoea types. In Figures 3 and 4, the proportion of the F4R− and F4R+ pigs from all five experiments on intervals of ln CFU PC_1 and % DM PC_1 are shown. In Figure 3, a decreasing value at the *x* axis of the ln CFU PC_1 plot means an increasing number of F4+ *E. coli* shed. As can be seen, the distributions of the F4R− and F4R+ pigs differed considerably along ln CFU PC_1; F4R+ pigs had a more equal distribution from low to high values whereas F4R− pigs were more concentrated on lower

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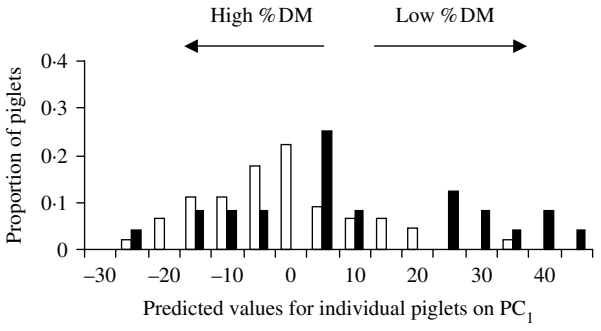

Fig. 4. Proportions of F4R+ (■, $n=24$) and F4R− pigs (□, $n=45$) (all experiments combined) on the predicted values of the percentage dry matter of faeces (% DM PC_1). Piglets with a predicted value of >11·65 were defined as having low % DM.

values of ln CFU PC_1 and thus on a lower number of bacteria shed. Only eight (17%) of the F4R− pigs were in the group of high shedders whereas of the F4R+ pigs 17 (71%) were in this group. Fisher's exact test for a 2×2 table results in a $P<0{\cdot}001$ thus showing that F4R status was highly associated with the classification into high and low shedders.

In Figure 4 an increasing value at the x axis of the % DM PC_1 plot means a decreasing overall mean and thus a lower % DM. As can be seen, there was considerable overlap between F4R+ and F4R− pigs, but F4R− pigs were more concentrated on a higher % DM. Applying Fisher's exact test on these results in a 2×2 table results in $P=0{\cdot}002$. So, although discrimination based on % DM PC_1 between the two diarrhoea types was not significant, the association of the F4R status with classification into high and low % DM clearly was significant.

The majority of pigs (61%) were assigned to both the low shedders and high % DM groups. Ten pigs (14%) were high shedders and in the low % DM group. Two pigs (3%) shed low numbers of F4+ *E. coli*, but were still classified into the low % DM group. Fifteen pigs were assigned to both the high-shedding group and the high % DM group. Analysing these results in a 2×2 table with Fisher's exact test, showed that ln CFU and % DM were significantly associated ($P<0{\cdot}001$).

DISCUSSION

Studies about PWD caused by F4+ *E. coli* have mainly focused on clinical symptoms of pigs, e.g. [9, 24], and not on the transmission of F4+ *E. coli*. However, to understand the epidemiology of PWD and for the development of control measures, information on the infectiousness and susceptibility of individuals to F4+ *E. coli* within a population is also needed. Shedding of F4+ *E. coli* in faeces may reflect differences in colonization and replication between pigs, and accordingly, differences in infectiousness. In this paper, we showed that by performing a PCA on the (log-transformed) F4+ *E. coli* shedding profiles of individual piglets, an objective linear discrimination measure could be derived that was able to classify these piglets into two significantly different groups. Although this statistical approach has been used in other or related fields [17–19], it has, as far as we are aware, never been described for finding measures of infectiousness. The shape of the eigenfunctions resulting from the PCA indicated that the main difference in shedding profiles of these two groups of piglets was in the total amount of bacteria shed at log-scale. We therefore referred to these groups as high- and low-shedding piglets. Whether the classification into high and low shedders reflects a classification into high and low infectiousness can only be studied in pigs housed together, because transmission is a process at the population level. Therefore, this measure for infectiousness was evaluated in a transmission experiment [25].

Besides the level of bacteria shed, other aspects like acid tolerance [26], survival of F4+ *E. coli* in faeces [27] and behaviour of the diseased pig [28] are likely to affect infectiousness. The degree of diarrhoea might also be a vector for spread of F4+ *E. coli*, since diarrhoeal faeces may form aerosols, although oral uptake might be less. Therefore, the faecal percentage dry-matter profiles of the pigs were also studied to find a measure to discriminate between diarrhoeic and non-diarrhoeic piglets. Large variation in % DM profiles was found between pigs, but the separation of the profiles into two distinct groups was not significant.

One of the factors that causes high variability in colonization and replication and thus in shedding and diarrhoea, is the presence or absence of the F4R. The presence of F4R was found to be associated significantly with low % DM. We also showed that the presence of F4R was significantly associated with high shedding of F4+ *E. coli* at log scale. This indicates that mixed F4R+/F4R− populations cannot be considered homogeneous with respect to shedding of F4+ *E. coli*. Therefore, F4R status of the pigs and its effect in populations will have to be taken into account in the evaluation of F4+ *E. coli* infection

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models or when testing F4+ *E. coli* intervention measures. Although the distributions of the F4R+ and F4R− pigs on the level of F4+ *E. coli* shedding at log scale had some overlap (Fig. 3), F4R seemed to be the major factor affecting the shedding profiles. This can be explained by the differences of specific adhesion to F4R on the brush borders of the pig [10] which is important with respect to colonization of F4+ *E. coli* [5, 7]. In ref. [11] it was also found that F4R affects the amount of bacteria shed, but in this study only separate days after weaning and not entire shedding profiles were investigated.

High shedders were classified more often in the group with a low % DM and low shedders in the group with a high % DM. However, 22% of the pigs (8 F4R+ and 7 F4R−) were both in the high-shedding and the high-percentage dry matter group. This indicates that compensatory mechanisms must be active to avoid fluid loss due to bacterial toxins both with F4R+ and F4R− pigs. Only two pigs were found in both the low % DM and low shedding group. These pigs might have suffered from rotavirus diarrhoea or other diarrhoeagenic causes.

In this study we wanted to find general measures to discriminate between infectious and non-infectious and between diarrhoeic and non-diarrhoeic piglets. We expected that the range in shedding profiles and percentage dry matter profiles of the five experiments together would best resemble the range in common pig practice. Therefore, we ignored the experiments and differences in experimental design as factors in the statistical analysis. The results gave no reason to think that differences in experimental design had major effects, except in experiment 5, where seven out of 11 F4R− pigs housed with F4R+ pigs were found in the high-shedding group. These pigs accounted for the majority of high-shedding F4R− pigs found in all experiments. This indicated that F4R+ pigs might have had an effect on the F4+ *E. coli* shedding of F4R− pigs. In this analysis we regarded the data from the pigs as independent data, but for the aforementioned pigs of experiment 5 this was not necessarily true. However, the relatively simple summary statistics of shedding and percentage dry matter derived in this paper gave robust results and showed the main differences between the individual profiles.

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DECLARATION OF INTEREST

None.

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