Epidemiol. Infect. (1999), **122**, 485–496. Printed in the United Kingdom

Sampling considerations for herd-level measurement of faecal *Escherichia coli* antimicrobial resistance in finisher pigs

R. H. DUNLOP[1], S. A. McEWEN*[1], A. H. MEEK[1], R. M. FRIENDSHIP[1], W. D. BLACK[2] AND R. C. CLARKE[3]

[1] *Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada N1G 2W1*
[2] *Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1*
[3] *Health of Animals Laboratory, Health Canada, 221 Stone Road W. Guelph, Ontario, Canada*

(*Accepted 25 January 1999*)

SUMMARY

The objective of this study was to determine the most efficient means of sampling faeces of finisher pigs for accurate and precise farm-level estimates of antimicrobial resistance among faecal *Escherichia coli*. Resistance to tetracycline and gentamicin of 8250 isolates of *E. coli* from 55 finisher pigs on one farm was measured with a hydrophobic grid membrane filter method. The between-pig, within-pen component of variance in resistance was large (97·5%), while between-pen, within-room and between-room components were small (2·5% and 0%, respectively). Using these resistance data, the abilities of two sampling strategies to estimate prevalence were modelled with a Monte Carlo 'bootstrap' procedure. Compositing faecal samples from several pigs before testing produced unbiased and precise estimates of prevalence and is simpler technically than individual animal testing.

INTRODUCTION

Reliable measurement of the abundance of antimicrobial resistant *Escherichia coli* in a pig population is dependent on the collection of a representative sample of the faecal *E. coli* population in the group of animals being studied [1]. Although sampling often accounts for the largest source of error in community level parameter measurement [2–4], it frequently receives insufficient consideration. Several authors have emphasized the importance of sampling in estimating antimicrobial resistant *E. coli* abundance [5–7], but few formal studies have investigated the effect of different sampling plans on the precision of resistance estimates in a population of animals. Hedges and colleagues [8] and Vosti and colleagues [9] developed and evaluated sampling plans for detecting *E. coli* serotypes within faecal specimens. However, these studies were not designed to estimate the abundance of these bacteria and the number or groupings of animals in the population were not considered.

In commercial pig operations, animals are usually grouped within pens, and these pens within rooms. Knowledge of the components of resistance variance that are attributable to various levels of organization should be obtained before sampling plans are designed. As cost and time constraints are often limiting [7, 10], there is a conflict between the number of isolates per specimen to be examined and the number of specimens to collect at each stage of nesting [11]. Resolving this conflict is important to achieving unbiased, precise estimates of antimicrobial resistant *E. coli* as the distribution of these bacteria in a pig population is heterogeneous [7, 12, 13].

The first objective of this study was to estimate the components of variance observed in antimicrobial resistant *E. coli* abundance at the level of individual pigs, pens and rooms within a farm. The second

* Author for correspondence.

objective was to use these data to evaluate the bias and precision of resistance prevalence estimates obtained by simulated individual and composite faecal sampling plans.

MATERIALS AND METHODS

An intensive, closed, farrow-to-finish swine operation with 300 sows was selected for the study because the pigs were individually identified, antimicrobial drug treatments were routinely recorded and no antimicrobials had been added to rations or water of grower-finisher pigs for the 3 years preceding the study; except that lincoymcin (44 g/t) was added to the rations of pigs up to 45 kg liveweight. Individual antimicrobial drug treatments were given to clinically ill pigs when necessary, mostly to suckling piglets and nursing sows.

Three of the 12 available pens, each housing about 10 healthy 75–100 kg liveweight finisher pigs were randomly selected from each of 2 rooms which were randomly selected from the 6 available rooms. A freshly voided 15 g faecal specimen was collected from each pig in selected pens using sterile tongue depressors and placed into a sterile plastic bag. Faecal specimens were processed as described previously (14). Faecal dry matter content was determined by weighing specimens before and after oven drying at 60 °C for 12 h [15].

Measurement of antimicrobial resistance

Tetracycline and gentamicin resistance were measured in this study because they were common and rare, respectively, in this and other populations of pigs [16, 17]. The breakpoint concentrations used (8 μg/ml tetracycline and 4 μg/ml gentamicin) were based on National Committee of Clinical Laboratory Standards (NCCLS) guidelines [18].

A hydrophobic grid membrane filter (HGMF) method, shown schematically in Figure 1, was used to estimate the concentration (number of *E. coli*/g faeces) and proportion of faecal *E. coli* resistant to 8 μg/ml tetracycline and 4 μg/ml gentamicin in each specimen. The method is described in detail elsewhere and was shown to be reliable and accurate [14, 16]. About 150 colony forming units (c.f.u.) from a freshly thawed subsample of faecal homogenate were inoculated onto a sterile HGMF using a spread filter (Richard Brancker Research Ltd, Ottawa, Ontario, Canada). Inoculated HGMFs were transferred onto pre-dried Tryptic Soy Agar (Difco Laboratories) with 1·5% wt/vol magnesium sulphate (TSAM) and incubated for 4 h at 35 °C to resuscitate injured cells. The HGMF was subsequently transferred onto MacConkey agar (MAC) and incubated for 18 h at 35 °C (MAC–HGMF). This MAC–HGMF was denoted the master HGMF.

All HGMF copies placed onto Mueller–Hinton agar (MHA) (Difco Laboratories) were incubated at 35 °C for 18 h. For identification of presumptive *E. coli*, HGMF copies were placed onto TBA and incubated at $44{\cdot}5 \pm 0{\cdot}3$ °C for 24 h in a water jacketed incubator (National Appliance Co., Portland, OR, USA). Previous studies showed that 97% of growths under these conditions were confirmed to be *E. coli* by the VITEK GNI®system (Vitek Systems, Hazelwood, MO, USA) [14, 16]. The proportion of *E. coli* colonies resistant to antimicrobial drug *a* was calculated as follows:

$$\mathrm{p}(a) = \frac{\mathbf{n}\mathrm{MH}a \cap \mathbf{n}\mathrm{TBA} \cap \mathbf{n}\mathrm{MH}}{\mathbf{n}\mathrm{TBA} \cap \mathbf{n}\mathrm{MH}}, \quad (1)$$

where

$\mathrm{p}(a)$ = proportion of *E. coli* colonies resistant to antimicrobial *a*

$\mathbf{n}\mathrm{MH}a$ = number of colonies on HGMF growing on MH agar containing drug *a*

$\mathbf{n}\mathrm{TBA}$ = number of colonies on first copy HGMF growing on TBA agar without drug

$\mathbf{n}\mathrm{MH}$ = number of colonies on last copy HGMF growing on MH agar without drug.

The *E. coli* concentration (number of *E. coli* c.f.u./g fresh faeces) was estimated from the number of *E. coli* that grew on TBA–HGMF, the volume of diluent applied to the master MAC–HGMF, the dilution factor and, if necessary, the faecal dry matter content.

Data handling and statistical analysis

E. coli concentrations were normalized using the transformation, $\ln(E.\ coli\ \text{concentration} + 100)$, but proportion data were not transformed. Logistic regression analysis was used to estimate pen and room effects on proportions of *E. coli* resistant, using GLIM version 3.77 [19].

Custom programmes (copies available on request) were written in DBase IV (Ashton Tate Corporation, Torrence, CA, USA) to simulate the different sampling plans, and means, standard deviations, medians, and 5% and 95% percentiles for these plans were estimated using STATISTIX software (Analytical

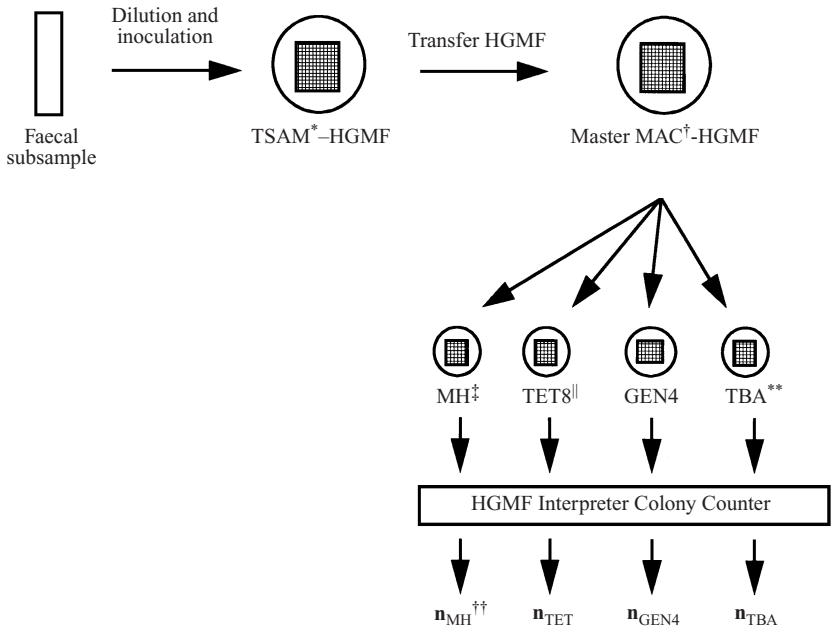

Fig. 1. Preparation of master HGMF from a faecal subsample and its replication and placement of HGMF copies on media for determination of proportion of *E. coli* resistant to tetracycline and gentamicin. Example calculation of proportion of *E. coli* resistant to antimicrobial a: $p(a) = (\mathbf{n}_{MHa} \cap \mathbf{n}_{TBA} \cap \mathbf{n}_{MH})/(\mathbf{n}_{TBA} \cap \mathbf{n}_{MH})$. *, Trypic soy agar with 1·5% wt/vol magnesium sulphate (TSAM) incubated at 35 °C for 4 h; †, MacConkey agar incubated at 37 °C for 18 h; ‡, Mueller–Hinton agar incubated at 35 °C for 18–20 h; ‖, Mueller–Hinton agar supplemented with tetracycline or gentamicin; **, Typtone bile agar incubated at 44·5 °C for 18–24 h; ††, number of colonies that grew on respective agar plate.

Software, St Paul, MN, USA). Probability density distributions were fitted to data using BESTFIT software (Palisade Corporation, Newfield, NY, USA).

The statistical distributions of abundance outcomes (i.e. proportions of *E. coli* resistant to antimicrobials and concentrations of *E. coli*) were described by fitting the normal, lognormal, logistic (proportion data) and beta (proportion data) probability density functions by using maximum likelihood techniques. The density function that resulted in the lowest value of the Anderson–Darling (AD) statistic was selected to describe the distribution of the outcome.

Pearson correlation coefficients were used to measure correlations between transformed *E. coli* concentrations and proportions of *E. coli* resistant to each antimicrobial. However, the low prevalence of gentamicin resistance precluded measurement of some correlations.

Pen and room effects on *E. coli* concentration and proportion resistant

Pen and room effects on *E. coli* concentrations were modelled as fixed effects using multiple linear regression techniques, while the proportions of *E. coli* resistant were modelled with logistic regression. The standard errors of the effect estimates from logistic regression were corrected for overdispersion of the residuals using Finney's correction factor [19]. Variable coding in pen effect regression models reflected that pens were nested within rooms, resulting in four degrees of freedom associated with the total sum of squares for the pen effect instead of five. Room effects were estimated after pen effect was removed from the model.

Measurements were made of the proportions of the variance of the transformed *E. coli* concentrations (both total and tetracycline-resistant) and the proportions of *E. coli* resistant to each antimicrobial that were attributable to the three levels of pig grouping. The between-pig within-pen, between-pen within-room and between-room components of variance were obtained using the random effects analysis of variance model with three stages of sampling [20].

Bootstrapping procedures for evaluation of sampling plans

Bootstrapping resampling techniques [21] were used to investigate the bias and precision of alternative

Table 1. *Distribution of measures of abundance of* E. coli *among 55 pig faecal specimens*

Measure of *E. coli* abundance	Mean	S.D.	Min.	Median	Max.	Skewness	Kurtosis
Faecal dry matter (%)	31·20	2·812	25·69	31·20	39·10	0·55	0·26
Number of isolates/specimen selected	150	72	45	136	394	1·39	2·06
E. coli concentration in fresh faeces ($\times 10^4$ c.f.u./g fresh wt)	384	790	8·27	102	4440	3·58	13·63
E. coli concentration in dried faeces ($\times 10^4$ c.f.u./g dry wt)	1280	2860	25·9	312	169000	4·05	17·41
Proportion tetracycline-resistant *E. coli* weighted by concentration	0·34	0·01	n.a.	n.a.	n.a.	n.a.	n.a.
Unweighted proportion *E. coli* resistant to tetracycline	0·41	0·27	0·01	0·36	0·96	0·49	−0·82
Concentration of tetracycline-resistant *E. coli* in fresh faeces ($\times 10^4$ c.f.u./g fresh faeces)	129	425	2·65	28·6	3110	6·50	42·79
Concentration of tetracycline-resistant *E. coli* in dry faeces ($\times 10^4$ c.f.u./g dry faeces)	450	1560	8·05	84·7	11400	6·51	42·86
Proportion gentamicin-resistant *E. coli* weighted by concentration	0·01	0·001	n.a.	n.a.	n.a.	n.a.	n.a.
Unweighted proportion of *E. coli* resistant to gentamicin	0·03	0·06	0	0	0·35	3·35	12·15
Concentration of gentamicin-resistant *E. coli* in fresh faeces ($\times 10^r$ c.f.u./g fresh faeces)	3·22	8·39	0	0	54·6	4·77	24·92
Concentration of gentamicin-resistant *E. coli* per g dried faeces (10^4 c.f.u./g dry faeces)	10·1	25·5	0	0	162	4·55	22·61

* n.a., not applicable.

sampling plans in estimating the farm-level proportions of faecal *E. coli* resistant to tetracycline and gentamicin. Bootstrapping can be used for non-parametric estimation of statistical parameters and it was selected for this study because prevalences were not normally distributed and one of the sampling plans involved two-stage sampling. The simulated sampling plans differed from one another in the method of selection of faecal specimens from the total of 55 available, and in the method of selecting *E. coli* from the specimens. Two parameters were varied; firstly, the number of faecal specimens collected (varying in resampling between 10 and 20), and secondly, the number of *E. coli* isolates selected from each specimen (varying in resampling procedures from 5–20).

Two sampling plans that were thought to be practicable alternatives for obtaining farm-level estimates of antimicrobial resistance were simulated; an individual faecal specimen plan and a composite plan. Conceptually, the individual plan comprised a simple random sample of n faecal specimens from which y/n colonies were randomly selected from each and tested for resistance. The composite simulated plan comprised a simple random sample of n faecal specimens from which equal volumes of faeces were composited. From this composited specimen, a random sample of y colonies of *E. coli* was randomly selected and tested for resistance.

The bootstrap resampling was carried out with the use of Monte Carlo simulation [21]. Briefly, 'n' observations were assumed to be independent and measured without error. These observations were used to randomly draw bootstrap samples with replacement, where each observation had an equal probability of selection. For the purposes of this study, the actual distribution of antimicrobial resistant *E. coli* within faecal specimens from 55 different pigs on one farm were used for resampling. The first step of bootstrapping consisted of randomly selecting the specified number of faecal samples using the appropriate sampling plan. The second step consisted of randomly selecting *E. coli* colonies using the appropriate sampling plan from selected faecal samples and assigning their antimicrobial resistance status (positive or negative) with probability prop-

ortional to the percent of resistant *E. coli* in the faecal specimen from which it originated. Proportions of resistant *E. coli* and concentrations of *E. coli* in the 55 faecal specimens that were used in bootstrapping were those obtained from the study farm and are described in Table 1.

RESULTS

A total of 8250 presumptive *E. coli* isolates were examined from 55 faecal specimens and descriptive statistics of antimicrobial resistance are described in Table 1. Overall, 41·0% and 2·8% of presumptive *E. coli* isolates were resistant to TET8 and GEN4, respectively. When weighted by the *E. coli* concentrations of faecal specimens, 33·5% and 0·8% of *E. coli* in the faeces of this population of finisher pigs were resistant to TET8 and GEN4, respectively.

Distribution of proportion of *E. coli* resistant to TET8 and GEN4

The proportion of *E. coli* resistant to TET8 was distributed between 0·1 and 1·0 (Fig. 2). The beta probability density function was a better fit to the observed proportions than the normal, logistic and log-normal probability density functions (AD = 0·28 *vs.* AD = 0·89, AD = 1·24 and AD = 1·28, respectively). The fitted parameter estimates of the beta probability function were $\alpha_1 = 0{\cdot}93$ and $\alpha_2 = 1{\cdot}41$. The fitted mean and standard deviation were identical to the unweighted observed population mean of 0·41 and standard deviation of 0·27 (Table 1).

The proportion of *E. coli* resistant to GEN4 followed the beta distribution (Fig. 3) better than the log-normal, logistic and normal probability density functions (AD = 4·03 vs AD = 5·76, AD = 9·96, AD = 10·18, respectively). The fitted parameter estimates of the beta probability function were $\alpha_1 = 0{\cdot}17$ and $\alpha_2 = 5{\cdot}72$. The fitted mean and standard deviation were identical to the unweighted observed population mean of 0·028 and standard deviation of 0·063 (Table 1). GEN4 resistant *E. coli* were not found in 28 of 55 specimens.

Distribution of *E. coli* concentration in faeces

Considerable between-faecal specimen variation in *E. coli* concentration was observed. However, *E. coli* concentration per g fresh faeces was highly correlated to concentration per g dry faeces ($r = 0{\cdot}998$, $P < 0{\cdot}0001$) (Table 1). Distributions of *E. coli* concentrations were skewed to the right (i.e. mean > median). The median total *E. coli* concentration was $1{\cdot}0 \times 10^6$ c.f.u./g fresh faeces, and ranged from $8{\cdot}3 \times 10^4$ to $16{\cdot}9 \times 10^7$ c.f.u./g fresh faeces. The log-normal probability density function fit the observed distribution of total *E. coli* concentration better than the normal probability functions (AD = 0·62 *vs.* AD = 10·05, respectively). The fitted estimates of mean and variance of the *E. coli* concentration in fresh faeces were $\mu = 3{\cdot}52 \times 10^6$ and $\sigma = 9{\cdot}76 \times 10^6$, respectively, which, in the case of the mean, was similar to the observed value of $3{\cdot}84 \times 10^6$ (Table 1).

The median concentration of *E. coli* resistant to TET8 was $0{\cdot}29 \times 10^6$ c.f.u./g fresh faeces, but ranged from $2{\cdot}7 \times 10^4$ to $3{\cdot}1 \times 10^7$ c.f.u./g fresh faeces. The log-normal probability density function described the observed distribution of this outcome better than the normal probability functions (AD = 0·46 *vs.* AD = 13·8, respectively), but it, too, was a poor fit to the data. The parameter estimates of the log normal function that best fit were $\mu = 9{\cdot}74 \times 10^5$ and $\sigma = 2{\cdot}48 \times 10^6$, which is similar to the observed $1{\cdot}29 \times 10^6$ c.f.u./g fresh faeces (Table 1).

The range of *E. coli* concentrations resistant to GEN4 was 0 to $54{\cdot}6 \times 10^4$ c.f.u./g fresh faeces. GEN4 resistant *E. coli* were not found in approximately a half of the specimens. Consequently, analysis of this outcome was not taken beyond evaluation of simple descriptive statistics.

Associations among proportion resistant and concentration of *E. coli*

A correlation matrix of proportions of *E. coli* resistant to antimicrobials and concentrations of resistant *E. coli* among the 55 faecal samples is presented in Table 2. The proportion of *E. coli* resistant to TET8 and GEN4 was not significantly correlated with the *E. coli* concentration of the specimen or the number of colonies tested per specimen ($r = -0{\cdot}2$, $P > 0{\cdot}1$). Similarly, the proportion resistant to TET8 was not significantly correlated with the proportion resistant to GEN4 ($r = -0{\cdot}023$, $P = 0{\cdot}87$). The proportion of *E. coli* resistant to GEN4 was significantly, but weakly, correlated with the concentration of TET8-resistant *E. coli* ($r = 0{\cdot}38$, $P = 0{\cdot}004$). However, the concentration of TET8-resistant *E. coli* was more highly

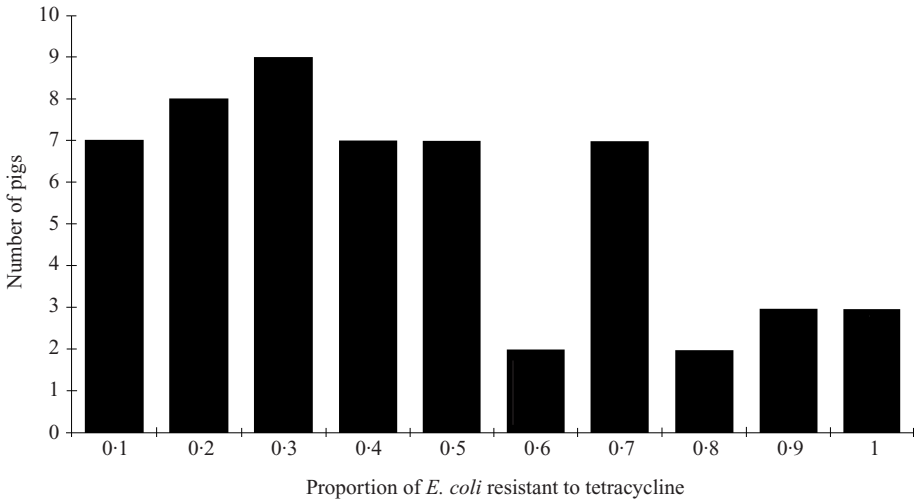

Fig. 2. Frequency distribution of the proportion of faecal *E. coli* resistant to 8 μg/ml tetracycline among a population of 55 finisher pigs.

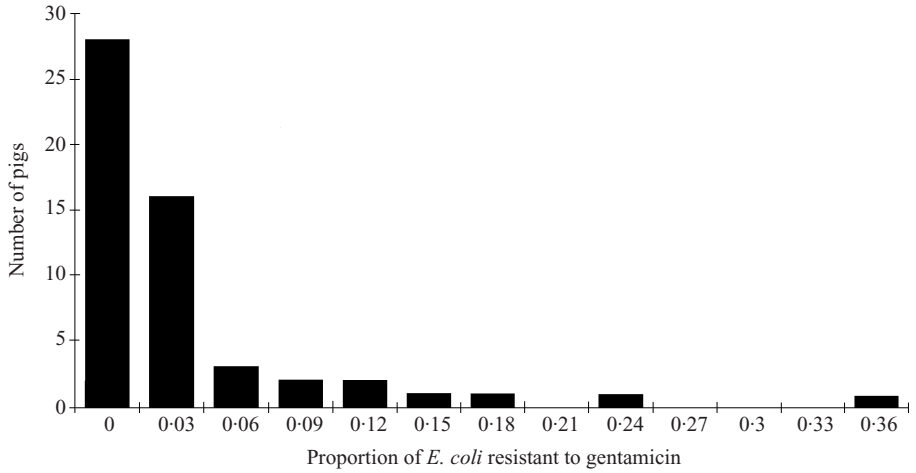

Fig. 3. Frequency distribution of the proportion of faecal *E. coli* resistant to 4 μg/ml gentamicin among a population of 55 finisher pigs.

correlated with the total *E. coli* concentration ($r = 0{\cdot}79$, $P = 0{\cdot}0005$).

Pen and room effects

The distribution of *E. coli* concentration in faeces of pigs by room and pen is presented in Table 3. The between-pig, within-pen component of variance of *E. coli* concentration was large (87·2%), while the between-pen, within-room and between-room components were small (12·8% and 0%, respectively). The differences in the *E. coli* concentration between pens were not significant ($P = 0{\cdot}07$), nor were the differences between rooms ($P > 0{\cdot}5$) or between sexes ($P = 0{\cdot}15$).

The distribution of *E. coli* resistant to TET8 in faeces of pigs by room and pen is presented in Table 4. The between-pig, within-pen component of variance of the proportion of *E. coli* resistant to TET8 was very large (97·5%), while the between-pen, within-room and between-room components were small (2·5% and 0%, respectively). On investigating pen and room effects using logistic regression analysis, overdispersion (clustering) was encountered, and the scale parameters used to adjust the likelihood ratio test statistic and standard errors of estimates were 47·26 and 6·87, respectively. The differences in the proportion resistant between rooms was not significant ($P > 0{\cdot}2$), nor were there sex differences ($P = 0{\cdot}14$).

The distribution of *E. coli* concentrations resistant to TET8 in faeces of pigs by room and pen is also

Table 2. *Pearson correlation coefficients and levels of significance among measures of faecal* E. coli *abundance from 55 finisher pig faecal specimens*

	Faecal *E. coli* abundance variable				
Variable name	ECOLI-F	ECOLI-D	CONCTET-F	CONCTET-D	PPNTET
ECOLI-D	0·998 (0·000)				
CONCTET-F	0·788 (0·000)	0·799 (0·000)			
CONCTET-D	0·779 (0·000)	0·793 (0·000)	0·998 (0·000)		
PPNTET	−0·197 (0·149)	−0·184 (0·179)	0·376 (0·005)	0·380 (0·004)	
PPNGEN	−0·217 (0·112)	−0·217 (0·112)	−0·191 (0·162)	−0·189 (0·167)	−0·023 (0·871)

* Variable name definitions: ECOLI-F, *E. coli* concentration per g fresh faeces; ECOLI-D, *E. coli* concentration per g oven dried faeces; CONCTET-F, concentration of tetracycline-resistant *E. coli* per g fresh faeces; CONCTET-D, concentration of tetracycline-resistant *E. coli* per g oven dried faeces; PPNTET, proportion of *E. coli* resistant to tetracycline; PPNGEN, proportion of *E. coli* resistant to gentamicin.
Note: All *E. coli* concentrations were transformed to the logarithmic scale using $\ln(E.\ coli + 100)$. Prior to estimating Pearson correlation coefficients (see Materials and Methods).

Table 3. *The distribution of* E. coli *concentration in faeces by room and pen among 55 finisher pigs*

Factor				*E. coli* concentration in faeces*				
Room	Pen	Sex	No. pigs	Mean	S.D.	Min.	Median	Max.
1	1	Female	8	13·72	1·46	11·90	13·74	16·41
	2	Male	8	14·14	0·92	13·11	13·75	16·04
	3	Female	10	14·04	1·401	12·06	13·98	16·30
	Room total		26	13·97	1·25	11·90	13·80	16·41
2	1	Male	10	13·16	1·94	11·32	12·18	16·02
	2	Female	10	15·03	1·69	13·03	14·46	17·61
	3	Mix	9	13·83	0·61	12·91	13·66	15·03
	Room total		29	14·01	1·69	11·32	13·86	17·61
	Combined total		55	13·99	1·48	11·32	13·83	17·61

* *E. coli* concentrations were transformed to log base e.

presented in Table 4. The between-pig, within-pen component of variance of *E. coli* concentration resistant to TET8 was large (79·0%) while the between-pen, within-room and between-room components were smaller (21·1% and 0%, respectively). Although there was significant between-pen, within-room variation in the concentration of *E. coli* resistant to TET8 ($P = 0{\cdot}02$), the differences were generally within 1 log of the overall mean (Table 4) and there was no evidence that TET8-resistant *E. coli* concentration differed significantly between rooms ($P > 0{\cdot}5$) or between sexes ($P = 0{\cdot}25$).

The distribution of *E. coli* resistant to GEN4 in faeces of pigs by room and pen is presented in Table 5; concentrations are not described owing to the absence of GEN4 resistance in approximately a half of the specimens. The between-pig, within-pen component of variance of the proportion of *E. coli* resistant to TET8 was very large (98%), while the between-pen, within-room and between-room com-

Table 4. *The distribution of* E. coli *resistance to 8 μg/ml tetracycline by room, pen and sex among 55 finisher pigs*

Factor				Proportion of *E. coli* resistant to 8 μg/ml tetracycline					Concentration of *E. coli* resistant to 8 μg/ml tetracycline*				
Room	Pen	Sex	No. pigs	Mean	S.D.	Min.	Median	Max.	Mean	S.D.	Min.	Median	Max.
1	1	Female	8	0·42	0·27	0·02	0·36	0·84	12·49	1·14	10·85	12·47	13·92
	2	Male	8	0·50	0·35	0·08	0·52	0·94	13·11	1·37	11·03	13·27	14·57
	3	Female	10	0·24	0·14	0·05	0·21	0·43	12·42	1·10	10·44	12·18	14·67
		Room total	26	0·37	0·27	0·02	0·35	0·94	12·65	1·19	10·44	12·36	14·67
2	1	Male	10	0·46	0·27	0·01	0·43	0·87	11·98	1·56	10·19	11·36	14·93
	2	Female	10	0·44	0·26	0·07	0·44	0·96	14·00	1·65	11·78	13·81	17·25
	3	Mix	9	0·42	0·29	0·09	0·29	0·84	12·70	0·93	11·37	12·56	14·05
		Room total	29	0·44	0·26	0·01	0·39	0·96	12·90	1·629	10·19	13·11	17·25
		Combined total	55	0·41	0·27	0·01	0·36	0·96	12·78	1·43	10·19	12·56	17·25

* *E. coli* concentrations were transformed to log base e.

Table 5. *The distribution of the proportion of* E. coli *resistant to 4 μg/ml gentamicin by room and pen among 55 finisher pigs*

Factor				Proportion of *E. coli* resistant to 4 μg/ml gentamicin				
Room	Pen	Sex	No. pigs	Mean	S.D.	Min.	Median	Max.
1	1	Female	8	0·02	0·03	0	0·01	0·07
	2	Male	8	0·02	0·04	0	0·01	0·11
	3	Female	10	0·03	0·07	0	0	0·22
		Room total	26	0·02	0·05	0	0·01	0·22
2	1	Male	10	0·06	0·11	0	0·01	0·35
	2	Female	10	0·02	0·04	0	0	0·11
	3	Mix	9	0·02	0·05	0	0	0·16
		Room total	29	0·03	0·07	0	0	0·35
		Combined total	55	0·03	0·06	0	0	0·35

ponents were small. The data were overdispersed on logistic regression analysis of pen and room effects, and the scale parameters used to adjust the likelihood ratio test statistic and standard errors of estimates were 13·55 and 3·681, respectively. The differences in the proportion resistant to GEN4 between rooms was not significant ($P > 0{\cdot}50$), nor were there significant differences between sexes ($P = 0{\cdot}23$).

Evaluation of resistance sampling plans by bootstrapping

The effect of faecal specimen and *E. coli* selection method on estimation of the proportion of *E. coli* resistant to 8 μg/ml tetracycline, using Monte Carlo bootstrap resampling, is presented in Table 6. The individual sampling plan produced normally distributed TET8 resistance prevalence estimates, while a right-skewed distribution was achieved with the composite sampling plan. Further, the individual sampling plan produced smaller standard deviations, but larger biases than the composite sampling plan for the same number of samples and colonies. Only small improvements in precision were observed when more than an average of five colonies per faecal specimen was examined (Table 6).

As with TET8 resistance, the individual sampling plan produced normally distributed GEN4 resistance prevalence estimates, while right-skewed distributions were achieved with the composite sampling plan. In contrast with TET8 resistance, however, the indi-

Table 6. *The impact of method of faecal specimen selection and* E. coli *colony selection from a population of 55 finisher pigs on estimation of the proportion of* E. coli *resistant to 8 μg/ml tetracycline, using Monte Carlo bootstrap resampling*

Faecal specimens	Colonies per specimen	Total colonies	Mean	S.D.	5th percentile	Median	95th percentile
Proportion of *E. coli* resistant to 8 μg/ml tetracycline							
Random sample of *E. coli* from composited faecal specimen							
10	5	50	0·34	0·206	0·100	0·28	0·800
10	10	100	0·34	0·200	0·100	0·28	0·770
20	5	100	0·34	0·160	0·140	0·29	0·680
20	10	200	0·33	0·164	0·150	0·28	0·660
20	20	400	0·33	0·160	0·160	0·28	0·655
Random sample of *E. coli* from individual faecal specimens							
10	5	50	0·410	0·0981	0·241	0·400	0·580
10	10	100	0·406	0·0920	0·251	0·400	0·560
20	5	100	0·411	0·0729	0·290	0·410	0·540
20	10	200	0·410	0·0658	0·295	0·410	0·515
20	20	400	0·406	0·0608	0·303	0·405	0·512
Proportion of *E. coli* resistant to 4 μg/ml gentamicin							
Random sample of *E. coli* from composited faecal specimen							
10	5	50	0·011	0·0187	0	0·000	0·060
10	10	100	0·013	0·0175	0	0·010	0·050
20	5	100	0·010	0·0139	0	0·010	0·040
20	10	200	0·010	0·0105	0	0·010	0·030
20	20	400	0·011	0·0103	0	0·008	0·032
Random sample of *E. coli* from individual faecal specimens							
10	5	50	0·030	0·0293	0	0·020	0·080
10	10	100	0·028	0·0250	0	0·020	0·080
20	5	100	0·028	0·0207	0	0·020	0·070
20	10	200	0·028	0·0184	0·0050	0·025	0·060
20	20	400	0·028	0·0162	0·0050	0·025	0·058

vidual plan yielded larger standard deviations, as well as larger biases of GEN4 prevalences than did the composite plan for the same numbers of samples and colonies selected. For the composite plan, only small improvements in precision were observed when more than an average of 10 colonies per sample were examined.

DISCUSSION

This study was unique in measuring the abundance of antimicrobial resistant *E. coli* in a large number of finisher pigs, reared under similar conditions. The variation in faecal *E. coli* concentration and abundance of antimicrobial resistant *E. coli* among individual finisher pigs was very large, as at least 80% of the total variance of these estimates was attributable to between-pig, within-pen effects. Variation between pens and rooms was less important, suggesting that emphasis should be placed on individual pigs, rather than pens or rooms, when sampling pig faeces on farms to determine *E. coli* antimicrobial resistance characteristics and concentration. The observed lack of substantial pen and room effects was applied to the bootstrapping studies of sampling strategies, in that pigs were considered to be independent of each other with respect to resistance and concentration of *E. coli* in faeces. The bootstrapping studies were useful in demonstrating that the choice of sampling plan can significantly influence bias and precision of antimicrobial resistant *E. coli* abundance estimates.

It should be emphasized that only one farm was used in this study and it would be wrong to generalize the results to the population of pig farms in Canada or

any other region. A strength of this study is the evaluation of resistance in large numbers of *E. coli* from a substantial number of individual pigs grouped to the pen and room level. Perhaps it would be useful to replicate the study in a larger number of farms with different management systems to judge whether conclusions from this study hold up.

Ideally, the various measures of abundance of antimicrobial resistance (i.e. proportion of isolates, concentration in faeces, proportion of positive animals) should be correlated with one another if results of studies using these measures are to be compared. The concentration and proportion of TET8-resistant *E. coli* were only weakly correlated. However, a large positive correlation was observed between *E. coli* concentrations in fresh and dried faeces ($r = 0{\cdot}99$). Therefore there seems little value in correcting faecal *E. coli* concentrations for faecal moisture content when healthy finisher pigs have been housed together and fed similar diets.

Accurate description of the distributions of public health-related events such as resistance abundance is important for making correct inference from statistical tests, and for conducting quantitative risk assessments of public health concerns where such data may be used as inputs where it is important to assign the correct distribution [22]. The distribution of *E. coli* concentration was better described by the log-normal probability density function than the normal probability density functions, whereas the distribution of proportion data was better described by the beta probability density function than the logistic, normal or log-normal probability density function. Previous studies have not described the probability distribution of resistance abundance, perhaps because the numbers of observations were insufficient to do so.

One might expect that pigs reared together in close confinement under similar conditions since birth would possess a similar gut flora with small pig–pig variation due to the constant exposure to each others' *E. coli* populations. In this study, however, the predominant component of variance of resistance concentration and prevalence was at the individual animal level, with only minimal variation due to pen or room effect. Similar wide ranges in the proportion of TET8-resistant faecal *E. coli* between animals housed together have been observed in unmedicated finisher pigs [23], unmedicated suckling and weaner pigs [10], and medicated weaner pigs [24]. Few studies have reported the variation of the proportion of GEN4-resistant faecal *E. coli* among pigs, but several studies have noted that less than 0·05 of porcine *E. coli* isolates were gentamicin-resistant [25, 26].

Pen effects were not significant in logistic regression in this study ($P = 0{\cdot}18$). In contrast, Hedges and colleagues [13] and Langlois and colleagues [27] reported large differences in the proportion of resistant *E. coli* between pens of pigs. It is questionable, however, whether true differences between pens existed in these studies given the sampling plans used.

Total *E. coli* concentrations varied by as much as 100-fold between pigs within the same pen and similar inter-individual variation has been reported in other studies [24, 25]. Although both total and TET8-resistant *E. coli* concentrations varied among pens ($P = 0{\cdot}07$ and $P = 0{\cdot}02$, respectively), pen means were all within 1 log (base e) unit from the overall mean, these differences are probably not of practical significance.

A bootstrap resampling approach was used in this study to investigate sampling issues at the levels of the faecal specimen and *E. coli* isolate because excessive laboratory resource demands precluded a comprehensive observational approach to the issue. The composite sampling plan is the simplest, easiest and least expensive way to obtain an estimate of antimicrobial resistance in a population of *E. coli* in the faeces of a group of pigs. Before using the method in a large observational study [28], however, it was desirable to compare its performance with a practicable alternative sampling method based on selection of a fixed number of isolates from individual faecal samples. The composite sampling plan is a type of informal probability proportional to size (PPS) plan, because the number of *E. coli* contributed by a specimen to the composite is a function of its *E. coli* concentration when equal weights or volumes are used.

For both sampling plans, the precision of the estimate of TET8 resistance was more sensitive to the number of faecal specimens collected than to the number of *E. coli* colonies selected per specimen, as the precision of estimates did not improve noticeably when more than an average of five *E. coli* colonies were selected per faecal specimen. This finding agrees with the general recommendation that only five elements need be selected per cluster for large multistage cluster samples [29].

Composite sampling is a type of weighted sampling, while the individual specimen method is not, hence it is not surprising that the prevalence estimates achieved by these bootstrapped sampling plans ap-

proached either the weighted (i.e. the 'true' prevalence) or unweighted observed prevalences. The individual specimen plan yielded a type of ratio estimate, and this study suggests that there may be considerable bias in such an estimate, particularly when the prevalence of the outcome is rare. The bootstrapping exercise also demonstrated the importance of between-pig variation on the precision of the prevalence estimate, and the value of weighting these estimates by the concentration of *E. coli*.

The results from the on-farm and bootstrapping studies help in designing a cost-efficient sampling plan for estimation of antimicrobial resistant *E. coli* in finisher pigs. Given the importance of between-pig variation in resistance, emphasis should be given to maximizing the number of individual animal samples in composites. Even though pen effects were not a substantial source of variation compared to the variation between pigs, this study was carried out on one farm only and it is therefore advisable to stratify collection of faecal specimens on the basis of finisher pig grouping (i.e. pen and rooms on most farms), weighting selection in proportion to the number of pigs in the pen.

As a measure of the abundance of antimicrobial resistant *E. coli* in faeces, there is a question of which should be promoted in future studies, the proportion of *E. coli* resistant to antimicrobial or the concentration of resistant *E. coli*? There are advantages to both, but the most useful all-round outcome appears to be the proportion resistant. In general, unweighted proportions have been more widely used as indices of abundance [17, 30] and are easier to explain than bacterial concentrations. The proportions of resistant *E. coli* are believed to be reasonably constant in dynamic pig populations [12]. Bootstrap resampling demonstrated that use of the proportion resistant can have a disadvantage, namely, that unweighted prevalence estimates are biased in terms of the 'true' cross-sectional prevalence of antimicrobial resistance in the faeces of a population of pigs. Consequently, the concentration of *E. coli* in faeces should also be reported to assist in conversion to concentration of resistance. This information may be needed by other workers for conducting quantitative microbial risk assessments or for other purposes. For example, a quantitative risk assessment model of pork contamination with resistant *E. coli* from faeces at slaughter would be enhanced by knowledge of the distribution of the concentration of resistant bacteria in faeces.

ACKNOWLEDGEMENTS

We are grateful to the swine producers of Ontario for participating in this study. Financial support was provided by the Ontario Ministry of Agriculture, Food and Rural Affairs, from Agriculture and Agri-Food Canada and from the Western Australia Department of Agriculture.

REFERENCES

1. Atlas RM, Bartha R. Microbial ecology; fundamentals and applications, 3rd ed. Redwood City, California: The Benjamin/Cummings Publishing Co., Inc., 1993.
2. Park DL, Pohland AE. Sampling and sample preparation for detection and quantitation of natural toxicants in food and feed. J Assoc Off Anal Chem 1989; **72**: 399–404.
3. Garfield FM. Sampling in the analytical scheme. J Assoc Off Anal Chem 1989; **72**: 405–501.
4. Jewers K, Coker RD, Jones BD, et al. Methodological developments in the sampling of foods and feeds for mycotoxin analysis. J Appl Bacteriol Symp Supp 1989: 105–16.
5. Corpet DE. An evaluation of methods to assess the effect of antimicrobial residues on the human gut flora. Vet Microbiol 1993; **35**: 199–212.
6. Wray C. Some aspects of the occurrence of resistant bacteria in the normal animal flora. J Antimicrob Chemother 1986; **18** (suppl. C): 141–7.
7. Walton JR. A dynamic study of drug resistance in populations of faecal *Escherichia coli* from pigs fed nitrovin and furizolidone continuously. Zbl Vet Med B 1972; **19**: 646–54.
8. Hedges AJ, Howe K, Linton AH. Statistical considerations in the sampling of *Escherichia coli* from intestinal sources for serotyping. J Appl Bacteriol 1977; **43**: 271–80.
9. Vosti KL, Monto AS, Rantz LA. The importance of sample size in studies based upon the serological classification of *Escherichia coli*. Proc Exper Biol Med 1962; iii: 201–4.
10. Hinton M, Hampson DI, Hampson E, Linton AH. The effects of oxytetracycline on the intestinal *Escherichia coli* flora of newly weaned pigs. J Hyg 1985; **95**: 77–85.
11. Kinkel LL, Nordheim EV, Andrews JH. Microbial community analysis in incompletely or destructively sampled systems. Microbial Ecology 1992; **24**: 227–42.
12. Linton AH, Hedges AJ, Bennett PM. Monitoring for the development of antimicrobial resistance during the use of olaquindox as a feed additive on commercial pig farms. J Appl Bacteriol 1988; **64**: 311–27.
13. Hedges AJ, Linton AH. Olaquindox resistance in the coliform flora of pigs and their environment: an ecological study. J Appl Bacteriol 1988; **64**: 429–43.
14. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Friendship RM, Black WD, Sharpe AN. Measuring antimicrobial-resistant *Escherichia coli* in pig faeces

using a hydrophobic grid membrane filter interpreter system. Appl Environ Microbiol 1998; **64**: 366–9.
15. Helrich K, ed. Official methods of analysis of the association of official analytical chemists. 15th ed. v. 1. Arlington: Association of Official Analytical Chemists, 1990.
16. Dunlop RH. Antimicrobial treatments and antimicrobial resistance of faecal *Escherichia coli* of swine in Ontario, Canada [PhD dissertation]. Guelph, Ontario: University of Guelph, 1996.
17. Langlois BE, Cromwell GL, Stahly TS, Dawson KA, Hays VW. Antibiotic resistance of faecal coliforms after long-term withdrawal of therapeutic and subtherapeutic antibiotic use in a swine herd. Appl Environ Microbiol 1983; **46**: 1433–4.
18. National Committee for Clinical Laboratory Standards. Approved standard M7-A2. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. Villanova Pa: National Committee for Clinical Laboratory Standards, 1990.
19. Aitkin M, Anderson D, Francis B, Hinde J. Statistical modelling in GLIM. Oxford: Clarendon Press, 1989.
20. Snedecor GW, Cochran WG. Statistical methods, 8th ed. Ames: Iowa State University Press, 1989.
21. Efron B. The jackknife, the bootstrap and other resampling plans. Philadelphia: Society for Industrial and Applied Mathematics, 1982.
22. Vose D. Quantitative risk analysis. A guide to Monte Carlo simulation modelling. Chichester; John Wiley & Sons, 1996.
23. Linton AH, Handley B, Osborne AD, Shaw BG, Roberts TA, Hudson WR. Contamination of pig carcasses at two abattoirs by *Escherichia coli* with special reference to O-serotypes and antibiotic resistance. J App Bacteriol 1976; **41**: 89–110.
24. Linton AH, Howe K, Osborne AD. The effects of feeding tetracycline, nitrovin and quindoxin on the drug resistance of Coli-aerogenes bacteria from calves and pigs. J Appl Bacteriol 1975; **38**: 255–75.
25. Langlois BE, Cromwell GL, Hays VW. Influence of chlortetracycline in swine feed on reproductive performance and on incidence and persistence of antibiotic resistant enteric bacteria. J Anim Sci 1978; **46**: 1369–82.
26. Hummel R, Tschape H, Witte W. Spread of plasmid-mediated nourseothricin resistance due to antibiotic use in animal husbandry. J Basic Microbiol 1986; **26**: 461–6.
27. Langlois BE, Dawson KE, Stahly TS, Cromwell GL. Antibiotic resistance of faecal coliforms from swine fed subtherapeutic and therapeutic levels of chlortetracycline. J Anim Sci 1984; **58**: 666–74.
28. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial treatments and antimicrobial resistance of faecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. Prev Vet Med 1998; **34**: 283–305.
29. Babbie E. The practice of social research, 5th ed. Belmont, California: Wadsworth Publishing Company, 1989.
30. Hinton M. The ecology of *Escherichia coli* in animals including man with particular reference to drug resistance. Vet Rec 1986; **119**: 420–6.