A survey of fluoroquinolone resistance in *E. coli* and thermophilic *Campylobacter* spp. on poultry and pig farms in Great Britain

Taylor, N. M.¹, Davies, R. H.², Ridley, A.², Clouting, C.², Wales, A. D.² and Clifton-Hadley, F. A.²

¹ Veterinary Epidemiology and Economics Research Unit, School of Agriculture, Policy and Development, University of Reading, Earley Gate, P.O. Box 237, Reading, Berkshire RG6 6AR, UK

² Department of Food and Environmental Safety, Veterinary Laboratories Agency - Weybridge, New Haw, Addlestone, Surrey KT15 3NB, UK

Correspondence:
Nick M. Taylor, Veterinary Epidemiology and Economics Research Unit, School of Agriculture, Policy and Development, University of Reading, Earley Gate, P.O. Box 237, Reading, Berkshire RG6 6AR, UK
Tel: +44 (0) 118 931 6633
Fax: +44 (0) 118 926 2431
Email: n.m.taylor@reading.ac.uk

Running title: Fluoroquinolone resistance on farms
ABSTRACT

Aims: To estimate the proportions of farms on which broilers, turkeys and pigs were shedding fluoroquinolone (FQ)-resistant *E. coli* or *Campylobacter* spp. near to slaughter.

Methods and Results: Freshly-voided faeces were collected on 89 poultry and 108 pig farms and cultured with media containing 1.0 mg l$^{-1}$ ciprofloxacin. Studies demonstrated the specificity of this sensitive method, and both poultry and pig sampling yielded FQ-resistant *E. coli* on 60% of farms. FQ-resistant *Campylobacter* spp. were found on around 22% of poultry and 75% of pig farms. The majority of resistant isolates of *Campylobacter* (89%) and *E. coli* (96%) tested had minimum inhibitory concentrations for ciprofloxacin of ≥8 mg l$^{-1}$. The proportion of resistant *E. coli* and *Campylobacter* organisms within samples varied widely.

Conclusions: FQ resistance is commonly present among two enteric bacterial genera prevalent on pig and poultry farms, although the low proportion of resistant organisms in many cases requires a sensitive detection technique.

Significance and Impact of Study: FQ-resistant bacteria with zoonotic potential appear to be present on a high proportion of UK pig and poultry farms. The risk this poses to consumers relative to other causes of FQ-resistant human infections remains to be clarified.

Keywords: pig, poultry, broiler, turkey, *Campylobacter*, *E. coli*, fluoroquinolone, resistance
INTRODUCTION

Fluoroquinolones (FQ) are a class of antimicrobial drugs effective against a wide range of human diseases and are used in both treatment and prophylaxis of bacterial infections. FQ have been particularly important in the treatment of serious food-borne infections which may often be resistant to other antimicrobials (Hopkins et al. 2005). FQ are also used in veterinary medicine and there is concern that their use in food animals may lead to resistance in certain bacteria, resulting in difficulty in treating food-borne infections in man (Aarestrup and Wegener 1999; van den Boogard and Stobberingh 2000; Bager and Helmuth 2001; Webber and Piddock 2001).

Quinolone and FQ antimicrobials were introduced, initially on a trial basis, in poultry and as quasi-legal imports from the Netherlands for veal production, followed by official licensing for poultry in the UK in 1993 (Piddock 1995). Indications for use in poultry extend to respiratory and enteric diseases and septicaemia (Hopkins et al. 2005). A rapid rise in resistance to the quinolone nalidixic acid was observed in Salmonella from turkeys following the introduction of FQ treatment (Davies et al. 1999). Resistance to FQ in human isolates of Campylobacter has been reported worldwide since the early 1990s (Endtz et al. 1991; Piddock 1995; Engberg et al. 2004), with increasing resistance in Escherichia coli documented more recently (Acar and Goldstein 1997; Threlfall et al. 1998). FQ resistance in human, veterinary and food isolates of Campylobacter and E. coli is reported commonly in surveillance (Anon 2005a, b; Bengtsson et al. 2005) and research (Giraud et al. 2001; Gupta et al. 2004; Ge et al. 2005) literature.

Thermophilic Campylobacter spp. are common commensal organisms of poultry and pigs, with zoonotic potential (Padungton and Kaneene 2003). Rapid selection of resistance has been observed following standard FQ therapy of Campylobacter–colonised broiler chickens under both natural (Griggs et al. 2005) and experimental (McDermott et al. 2002; van Boven et al. 2003) conditions, and in treated pigs (Delsol et al. 2004). Prior to veterinary licensing of FQ, levels of resistance amongst human and poultry Campylobacter were low (Endtz et al. 1991; Gaunt and Piddock 1996), and it has been proposed (Endtz et al. 1991) that the veterinary use of FQ has led to the selection of FQ-resistant Campylobacter, particularly in poultry, which then enter the food-chain and create a risk to public health. After the licensing of veterinary FQ, there was a substantial rise in FQ resistance among both veterinary and human Campylobacter isolates (Endtz et al. 1991; Aarestrup and Wegener 1999; Gupta et al. 2004) although in several countries an increasing trend was present before veterinary use, which often was associated with recent foreign travel (Rautelin et al. 1991; Smith et al. 1999).

The contribution of poultry meat to the transmission of resistant Campylobacter to humans has been vigorously debated. Consumption of meat products, particularly out of the home, is associated with an increased risk of infection with an FQ-resistant Campylobacter sp. (Engberg et al. 2004; Kassenborg et al. 2004), and there is evidence to support a substantial prevalence of FQ-resistant Campylobacter in poultry meat products (Endtz et al. 1991). However, foreign travel and swimming have also been identified as important risk factors (Engberg et al. 2004). The level of resistance appears to be related to the frequency of use of FQ (Padungton and Kaneene 2003). Thus, resistance to FQ among bacteria is higher in areas such as the Mediterranean (Baquero 1996) where FQ use is relatively high. Conversely, levels of FQ usage and of resistance amongst Campylobacter spp. in Scandinavian countries are typically low (Anon 2005a; Bengtsson et al. 2005). In Scandinavia, quinolone-resistant Campylobacter infections are often acquired by people during foreign travel (Sjogren et al. 1992; Norstrom et al. 2006) and in the UK and Scandinavia consignments of imported poultry meat have been identified as sources (Gaunt and Piddock 1996; Anon 2005a).

E. coli is a ubiquitous gut commensal organism with zoonotic potential but concerns in this regard have focused on verotoxigenic E. coli O157, for which antimicrobial therapy is usually contraindicated, and to a lesser extent on other toxigenic strains. There is, however, much evidence suggesting that E. coli strains which are primarily classed as commensals in farm livestock may
colonise the human intestinal tract (Linton 1977; Achtman et al. 1986; Cherifi et al. 1991, 1994) and human infection by antibiotic-resistant *E. coli* can be acquired by contact with farm animals (Levy et al. 1976; Linton 1977; Nijsten et al. 1994; Aarestrup and Wegener 1999). Further spread of these organisms within local communities has also been demonstrated (reviewed by Aarestrup and Wegener 1999). *E. coli* from animals found as contaminants on food can also colonise man (Cooke et al. 1970; Linton 1986).

FQ resistance in human *E. coli* isolates from blood and cerebrospinal fluid has been increasing in the UK since 1991 (Threlfall et al. 1998). Furthermore, *E. coli* FQ resistance appears to be especially marked in parts of the world with fewer constraints on the use of antimicrobials, such as some Mediterranean countries and the Middle East (Amara et al. 1995; Al-Mustafa and Al-Ghamdi 2000). However, the transfer of FQ resistance from veterinary strains of *E. coli* in contaminated food to human strains in the intestinal tract is assumed to be a low risk, as quinolone resistance is considered typically to be carried on the bacterial chromosome rather than on plasmids (Zhang et al. 2003), and experimental work in chickens (van Boven et al. 2003) has shown a lower propensity for selection of FQ resistance for *E. coli* than for *Campylobacter jejuni*. This may change in future as a human isolate of *E. coli* possessing a conjugative plasmid encoding a *qnr* determinant conferring low level resistance to quinolones has been reported for the first time in Europe (Mammeri et al. 2005).

The majority of antimicrobial products sold for use in food animals in the UK are authorised for pigs and poultry (http://www.vmd.gov.uk/Publications/Antibiotic/AntiPubs.htm) and bacteria in such systems might therefore be expected to exhibit relatively high levels of resistance to the antibiotics used including fluoroquinolones. The nature of the breeding pyramids and short production cycles mean that, depending on the mode(s) of the transfer of resistance, the impact of FQ resistance could spread rapidly through commercial units. In view of the foregoing, the present study examined pig and poultry units for FQ resistance, focusing on *Campylobacter* and *E. coli*. The latter bacterium was chosen both in view of its zoonotic potential and, being a ubiquitous representative of the *Enterobacteriaceae*, as an indicator species particularly for *Salmonella*. The irregular occurrence and clonal distribution of salmonellas independent of antimicrobial usage makes representative studies of *Salmonella* more difficult to perform. In the present studies, the prevalence of FQ resistance was examined at the farm level, and data were collected concurrently for risk factor analyses, which will be reported elsewhere.
MATERIALS AND METHODS

Recruitment for the surveys

**Poultry.** The target population for the survey was defined as: ‘poultry meat (broiler and turkey) farms in England and Wales with at least 1,000 birds on site’. This population, of 960 holdings, accounted for 99.9% of poultry meat (broiler and turkey) birds according to the MAFF June census of England and Wales in 1999. Most of the study population was recruited by approaching the relatively small number of large integrated poultry companies in England and Wales. Companies associated with 224 sites (23% of the estimated 960 large poultry sites in England and Wales), two of which were turkey companies, agreed to take part in the survey. Participating companies supplied complete lists of their poultry sites, which were used as sampling frames from which approximately one third of each company’s sites were randomly chosen for inclusion. To ensure representation of independent growers, additional recruitment was done by advertising in a trade magazine. All eligible independent growers that responded were included in the survey.

**Pigs.** The target population was defined as: ‘pig finishing (breeding to finishing, or growing and finishing) farms in England, Scotland and Wales, with at least 100 breeding females if breeder to finish, or 200 finisher places if specialist finishers’. There were 2,650 eligible holdings in Great Britain according to the June 2002 Agricultural Census. Four hundred and sixteen pig farms, selected at random from lists provided by Quality Meat Scotland (QMS), Assured British Pigs and the National Pig Association, were contacted by their organisations and invited to join the study.

Organisation of field work and sampling protocols.

Poultry survey work was carried out between June 2001 and June 2003. Sampling and data collection on farms was done by either company-appointed poultry veterinarians or poultry company staff under the supervision of the company veterinarian. The independent poultry producers carried out the sampling and data collection themselves. Pig survey work was carried out between December 2002 and October 2003. Sampling and data collection on pig farms was carried out by the farm manager and the farm’s private veterinarian.

A protocol for sampling was devised, to provide a sensitivity of about 95% for the detection of resistant bacteria being shed on a unit. Assuming a minimum prevalence of 5% of animals on an affected farm shedding resistant bacteria and also assuming 90% sensitivity of the laboratory detection method, calculations based on binomial probabilities showed that 95% sensitivity could be achieved by taking samples from 64 animals into eight pools.

On each poultry farm, 64 separate fresh floor droppings were picked up from the litter from random locations in up to four houses. The samples were pooled into eight pots, each containing eight droppings samples. On pig farms, to meet the requirements of concurrent research projects, up to 30 faeces sample pools were created in pots. Each pot contained mixed faeces from at least eight animals. Eight pots were randomly selected for use in the present study. Sampling was targeted on birds or pigs near to slaughter, therefore houses containing the oldest birds, and only pens containing finishing pigs were chosen for sampling. Each pot was stirred with a fresh transport medium swab for the preservation of *Campylobacter*. The pots and swabs were transported to the laboratory by post. Background data on the farm, including FQ use, were collected using questionnaires completed by the farm manager and/or the attending veterinarian.

Laboratory processing of the samples

For isolation of *E. coli*, 1 g of faeces from each pool was mixed with 9 ml buffered peptone water (BPW; Oxoid), and 100 μl aliquots of each suspension were spread over the surface of chromogenic
E. coli / coliform agar plates (Chromagar ECC; CM956, Oxoid) containing 1.0 mg l\(^{-1}\) ciprofloxacin, and incubated overnight at 37\(^\circ\)C. Plates streaked with control E. coli were incubated under the same conditions. Controls comprised one strain sensitive to ciprofloxacin, NCTC 10418 (National Collection of Type Cultures, Colindale, London, UK), and three strains from the VLA culture archive with known minimum inhibitory concentrations of 0.5 mg l\(^{-1}\) (F3), 1.0 mg l\(^{-1}\) (F15) and 2.0 mg l\(^{-1}\) (F13). The BPW suspensions were also incubated at 37\(^\circ\)C overnight. If the primary culture plates produced no growth, Chromagar ECC plates with ciprofloxacin were then inoculated with the pre-enriched sample in BPW and incubated overnight.

For isolation of thermophilic Campylobacter spp., transport swabs inoculated with pooled faeces (eight swabs per unit) were first streaked onto 10\% (v v\(^{-1}\)) sheep blood agar plates containing Skirrow’s antibiotic supplement (vancomycin, 10 mg l\(^{-1}\); polymyxin B, 2500 iu l\(^{-1}\); trimethoprim, 5 mg l\(^{-1}\); actidione, 250 mg l\(^{-1}\); and cefoperazone 15 mg l\(^{-1}\) (BASAC) to which 1.0 mg l\(^{-1}\) ciprofloxacin had been added, for primary culture. Control plates were streaked with one strain of C. jejuni sensitive to ciprofloxacin, NCTC 81116 (National Collection of Type Cultures, Colindale, London, UK), and one ciprofloxacin-resistant strain from the VLA culture archive (VLA 00/051). All the swabs from each unit were then pooled in 10 ml Exeter broth [Bolton broth (27.6 g l\(^{-1}\), CM983 Oxoid Ltd, Basingstoke, UK), Campylobacter growth supplement (sodium metabisulphate, sodium pyruvate and ferrous sulphate, all at 250 mg l\(^{-1}\); SV61 Mast diagnostics, Bootle, UK), Campylobacter selective supplement (trimethoprim, 10 mg l\(^{-1}\); rifampin, 5 mg l\(^{-1}\), polymyxin B, 2500 iu l\(^{-1}\); cefoperazone, 15 mg l\(^{-1}\); amphotericin B, 2 mg l\(^{-1}\); SV59 Mast Diagnostics) and defibrinated horse blood (10 ml l\(^{-1}\); TCS Biosciences Ltd, Botolph Claydon, UK)]. The BASAC and Exeter broth were incubated for 48 h at 37\(^\circ\)C in a microaerophilic (6\% O\(_2\), 10\% CO\(_2\)) incubator (Heraeus). After 48 h incubation, 50 \(\mu\)l aliquots of the inoculated Exeter media were streaked onto each of a BASAC plus ciprofloxacin (1.0 mg l\(^{-1}\)) and a plain BASAC plate, which were incubated for 24 to 48 h microaerophilically at 37\(^\circ\)C.

The growth of colonies typical of E. coli or Campylobacter on media containing ciprofloxacin (1.0 mg l\(^{-1}\)), where growths of ciprofloxacin- sensitive and ciprofloxacin-resistant control strains on the media were as expected, was taken to indicate that FQ-resistant bacteria were present in the faeces in the pot and therefore the farm of origin was classed as ‘affected’. The identity of putative E. coli colonies was confirmed using standard o-nitrophenyl-beta-galactopyranoside and indole tests with inconclusive results checked by API 20E (bioMérieux (UK) Ltd, Basingstoke, Hampshire). Colonies showing morphology typical of Campylobacter on BASAC were identified using standard oxidase, catalase, indoxyl acetate (CAMP 1A Kit; Mast Diagnostics) and hippurate tests. Representative E. coli isolates from each positive sample were stored on Dorset egg slopes and used in further investigations. Campylobacter spp. isolated from BASAC with and without ciprofloxacin were stored in 10\% glycerol broth at minus 80\(^\circ\)C for comparative typing investigations.

**Estimation of relative proportions of resistant bacteria**

E. coli. Aliquots (100 \(\mu\)l) of serial dilutions of faeces pools in phosphate-buffered saline were spread-plated onto paired Chromagar ECC plates with and without 1.0 mg l\(^{-1}\) ciprofloxacin and colonies typical of E. coli were counted after overnight incubation.

Campylobacter. Aliquots (100 \(\mu\)l) of pooled faeces of positive samples from 5 pig farms which had been serially diluted in Exeter broth and incubated microaerophilically for 48 h at 42\(^\circ\)C were spread-plated onto BASAC plates, with and without 1.0 mg l\(^{-1}\) ciprofloxacin, and Campylobacter colonies counted following incubation at 42\(^\circ\)C under microaerophilic conditions for 48 h.

The proportions of the E. coli and Campylobacter populations that were resistant were calculated by comparison of colony counts on ciprofloxacin and non-ciprofloxacin plates.
Minimum inhibitory concentration (MIC) of ciprofloxacin

Using the ciprofloxacin-resistant *E. coli* recovered from farms, as described above, MIC values to ciprofloxacin were determined by an agar doubling dilution method similar to that of the Clinical and Laboratory Standards Institute (NCCLS, 1997), with the main difference being that iso-sensitest agar (CM471 Oxoid Ltd) rather than Mueller Hinton agar was used. The reference strains for quality control were *E. coli* ATCC 25922 (NCCLS, 1997) and three strains from the VLA culture archive with known minimum inhibitory concentrations of 0.5 mg l\(^{-1}\) (F3), 1.0 mg l\(^{-1}\) (F15) and 2.0 mg l\(^{-1}\) (F13). Briefly, bacteria grown overnight at 37\(^\circ\)C in BPW were diluted 1/100 in peptone water and inoculated, using a multi-point inoculator, onto the agar with suitable dilutions of ciprofloxacin. Plates were incubated overnight at 37\(^\circ\)C and the MIC was recorded as the lowest concentration of ciprofloxacin inhibiting growth.

A selection of 24 and 33 ciprofloxacin-resistant *Campylobacter* isolates recovered from pigs and poultry respectively were evaluated by the NCCLS agar dilution method as described by Randall et al (2003). Briefly, the inoculum was prepared by growing the strains for 48 h on BASAC. Growth was emulsified in saline to a density approximating to McFarland No. 0.5 turbidity standard and used to inoculate Mueller Hinton agar (CM337 Oxoid Ltd) plates supplemented with 5% haemolysed horse blood and standardised concentrations of ciprofloxacin, using a multi-point inoculator. Inoculated plates were incubated at 37\(^\circ\)C in a variable atmosphere incubator (VAIN; Don Whitely Scientific) at H\(_2\) 3%, CO\(_2\) 5%, O\(_2\) 5%, N\(_2\) 87% for 48 h with the humidity adjusted to 50%. The MIC was taken as the concentration of ciprofloxacin that caused inhibition of growth by more than 90%. Control strains comprised *Campylobacter fetus* subsp. *fetus* NCTC 10842, *C. jejuni* NCTC 11168, *C. NCTC 11351, Campylobacter lari* NCTC 11352 and *Campylobacter coli* NCTC 11366.

Statistical analysis

To account for over-representation of certain strata within the poultry sample, weighting factors were used in the estimation of prevalence of affected farms in the target population. Weighting factors were not used in the analysis of pig data, as the study population closely matched the target population in respect of geographic distribution and representation of breeder versus grower units. Ninety five percent confidence intervals (CI\(_{95}\)) for the national prevalences of affected units were calculated using EpiInfo version 6 (Centers for Disease Control and Prevention U.S.A. & World Health Organisation, Geneva, Switzerland).
RESULTS

Participating farms

A total of 89 poultry farms were sampled and included in the analysis. The sample contained distinct strata, depending on type of ownership and type of end product. Independent growers and turkey farms accounted for 22% and 24% of the farms sampled respectively. These sectors were both over-represented, as the MAFF June census of England and Wales in 1999 gave figures of 10% for independents and 17% for turkey farms. The use of FQ (past and/or present) was reported on 25% of poultry farms.

Amongst pig units, the final sample used in the analysis was of 108 farms. The population of sampled farms generally matched the geographical distribution of the national herd. The only major stratification of farm type was breeder/finisher versus grower/finisher. Breeding farms made up 55% of the sample, compared with 59% of eligible GB holdings which had breeding pigs (June 2002 Agricultural Census). The use of FQ (past and/or present) was reported on 66% of pig farms.

Correlation between FQ resistance and use of these antimicrobials on farms will be reported elsewhere as part of a detailed risk factor analysis.

Prevalence of affected farms

The sample prevalences of farms where FQ-resistant organisms were detected, and the estimates of the prevalence in the target population (weighted in the case of poultry) with exact binomial CI95 are shown for Campylobacter (Table 1) and E. coli (Table 2).

Enrichment was required to grow FQ-resistant E. coli from samples from 37% (23 of 63) of the pig farms where resistant E. coli were detected, but from only 6% (3 of 53) of similarly affected poultry farms. In contrast, enrichment was required to grow FQ-resistant Campylobacter from samples from only 4% (3 of 81) pig farms where resistant Campylobacter was detected, but from 30% (6 of 20) of similarly affected poultry farms.

The distributions of resistant organisms isolated from farms are summarised in Fig. 1. Resistance in both bacterial species was common amongst pigs (52% of farms) and turkeys (38% of farms), but much less common in broilers (7% of farms). More pig farms had resistance in Campylobacter than in E. coli, and most with resistant E. coli also had resistant Campylobacter. Conversely, more poultry farms had resistance in E. coli than in Campylobacter. All of the turkey farms with resistant Campylobacter also had resistant E. coli.

Proportions of resistant bacteria

Bacterial population assessments for FQ-resistant versus total E. coli were carried out on 23 pooled faeces samples from each of eight poultry and seven pig farms affected by FQ resistance (Fig. 2). A wide range of proportions of FQ-resistant E. coli was found, with ranges of 0.00045% to 37% for poultry, and 0.0079% to 53% for pigs.

From five pig farms, 19 samples containing FQ-resistant Campylobacter were examined to estimate the proportion of resistant Campylobacter cells in the population. These proportions fell within a wide range, both within and between farms, of <10% to 100%. Estimations of resistant Campylobacter proportions were not carried out on any poultry samples.
Minimum inhibitory concentrations of ciprofloxacin

Of the 235 resistant *E. coli* tested, 226 were resistant to ciprofloxacin at concentrations >8 mg l⁻¹, and the remainder at 2 to 4 mg l⁻¹. For *Campylobacter* spp., all 33 isolates from poultry recovered on 1 mg l⁻¹ ciprofloxacin media and susceptibility tested had MIC values to ciprofloxacin of ≥8 mg l⁻¹ (range 8-128). Three of 24 pig isolates from 1 mg l⁻¹ ciprofloxacin media examined had MIC values of 2 mg l⁻¹, three had an MIC of 4 mg l⁻¹ and the rest had MIC values of ≥8 mg l⁻¹. All control strains gave the anticipated results.
DISCUSSION

The primary aim of the present survey was to estimate the proportion of poultry and pig farms in the target populations that were affected by FQ-resistant bacteria. The precision of this estimate is determined solely by the number of farms in the sample. The percentages of ‘affected’ farms presented in tables 1 and 2 have CI95 of about +/- 10%, with the exception of the estimates for the turkey farm stratum alone, which are +/- 20%. The accuracy of the estimated affected proportion of farms in the target population is influenced by the sensitivity and specificity of the detection method, and by any bias in the sample.

A low sensitivity of detection would lead to under-estimation. Several factors can affect sensitivity, in particular the on-farm sampling strategy, the effect of transit conditions on the samples and the sensitivity of the laboratory method. The sampling strategy was designed to give a 95% probability (i.e. sensitivity) of retrieving resistant bacteria on an affected farm, provided that a minimum of 5% of animals were shedding such bacteria. The effects of transit conditions on the samples are likely to have been variable, depending on the species of bacteria, and various sample transit factors such as time and temperature. Because Campylobacter is generally thought to be less robust than E. coli, detection sensitivity may have been lower for the former. For this reason, transport swabs were used for portions of the pools subjected to culturing for Campylobacter.

When devising the cultural methods, the primary concern was for sensitive detection of ‘affected’ farms (i.e. to minimise false negative results). The classical methods of plating on non-selective media followed by sub-culturing and testing of a ‘sub-sample’ of colonies for FQ resistance, or of replica plating, may not have been sufficiently sensitive. It was decided, therefore, to use antibiotic-supplemented media in the primary isolation plates. Enrichment was performed in parallel, to maximise the probability of recovery from samples with low concentrations of viable target bacteria. Ciprofloxacin at 1 mg l⁻¹ was used in all plates as it was considered that stressed organisms plated onto an inhibitory selective medium (BASAC) containing a higher level of ciprofloxacin may result in reduced growth of resistant organisms, increasing the likelihood of false negative results. MIC values were then determined for a selection of potentially resistant isolates to establish their level of resistance.

The finding that the proportion of the tested E. coli population resistant to FQ could be as low as approximately 5 in 10⁶ indicates the value of using the isolation approach described here. With such low numbers of resistant organisms, it is unlikely that they would have been detected by non-selective primary isolation and subsequent sub-culture. In addition to direct plating, enrichment of samples prior to plating helped to improve detection of low numbers of resistant organisms, particularly for Campylobacter spp. where six of the 18 positive poultry farms were detected only following enrichment of the pooled sample. A large majority of E. coli (96%) and Campylobacter (89%) isolates that were tested following isolation on ciprofloxacin-containing media had MIC values greater than 8 mg l⁻¹. Thus, the methods are considered to have been successful in identifying fully-resistant organisms, even when present in low numbers amongst a background of non-resistant members of the same species. Therefore the current results do not necessarily represent a sudden increase in the prevalence of affected farms compared with that reported previously; the higher prevalences reported here are more likely due to the detection method employed being more sensitive to a low prevalence of resistant organisms than are conventional sub-culturing or replica plating approaches.

A potential risk with highly sensitive detection methods is a lack of specificity. This would result in the incorrect classification of farms as ‘affected’ (false positive results) with a consequent over-estimation of the proportion affected. The use of ciprofloxacin in primary isolation plates reduced the work required to identify resistant organisms and improved the sensitivity of the detection method over the classical two-stage approach. However, the inclusion of 1 mg l⁻¹ ciprofloxacin in culture media potentially could select resistant bacteria spontaneously mutating in
culture, 90% of these farms still proved negative for resistant mutation. Furthermore, where enrichment was performed for farms negative on direct plating, resistant mutation rate was consistently at the highest end of the ranges quoted. In fact, the 91% of positive Campylobacter strains from a background of second-stage mutants with low-level FQ resistance of debatable significance. However, the mutation rate assumption used is rather generous, as measured rates for E. coli have been in the range of one per $10^7$ to $10^8$ in the face of media containing 2 to 16x the MIC of a FQ (Kern et al. 2000), so even with enriched inocula the theoretical possibility of selecting spontaneous mutants occurring after sampling is low.

MIC values of $\geq 2$ mg l$^{-1}$ ciprofloxacin among E. coli typically are seen after two or more stages of selection in vitro (Kern et al. 2000), and it is possible that the detection method employed in the present study would have selected second-stage resistant E. coli, with multiple mutations in topoisomerase and efflux genes, from a background of first-stage mutants with low-level FQ resistance of debatable significance. However, the mutation rate to second-stage resistance has been shown to be of the order of one per $10^8$ first-stage mutants plated (Kern et al. 2000) and therefore, similar to the discussion above, it remains highly unlikely that the observed results are a consequence of this and not of the pre-existence on the premises of resistance at clinically-significant MIC levels.

Considering Campylobacter, single-stage mutation to grow on media containing ciprofloxacin at 1-2 mg l$^{-1}$ has been reported to occur in C. jejuni at a rate of around 1 per $10^8$ cells plated (Gootz and Martin 1991) and more recently at a rate of between 7 per $10^3$ and 4 per $10^5$ cells plated (Hanninen and Hannula 2007). Under normal circumstances, measured concentrations of Campylobacter in pig caecal contents and broiler faeces are around $10^4$ to $10^8$ cfu g$^{-1}$ (Harvey et al., 2001; Stern and Robach 2003). Therefore, even with inocula of 1 g faeces per plate, i.e. significantly more than the swab inoculation used in the present study, very few plates (probably fewer than one in 100) would have grown a spontaneously ciprofloxacin-resistant Campylobacter sp. unless the mutation rate was consistently at the highest end of the ranges quoted. In fact, the 91% of positive farms that did not require enrichment for detection yielded resistant Campylobacter from a median of three (of eight) plates. Furthermore, where enrichment was performed for farms negative on direct culture, 90% of these farms still proved negative for resistant Campylobacter. The above considerations indicate that the incidental detection of post-sampling spontaneous FQ-resistant Campylobacter mutants was very likely to have been of negligible significance in the present work.

The accuracy of the estimate for the proportion of ‘affected’ farms in the target population is affected by the correlation between the sample and the population from which it is drawn. If the sample is in some ways biased compared to the target population (e.g. for farm types) the sample proportion may not be an accurate indicator of the population proportion. In Tables 1 and 2, adjustments to the results were made to account for over-representation of turkey farms and independent broiler farms in the poultry sample. This should allow more accurate inferences to be made about the wider population of poultry farms. However, such inferences must also assume that there were no significant biases between the poultry companies that were included and those that were not. Differences in the proportion of farms with resistant bacteria were seen between the
companies included in the survey (data not shown) and so it is not possible to assume that the companies included and those not included in the survey were highly similar. Therefore, inferences about the prevalence of shedding of FQ-resistant organisms in the general population of poultry farms should be treated with caution. However, animals were shedding resistant bacteria on a high proportion of farms and, in view of the wide confidence intervals (+/- approximately 10%), it seems reasonable to assume that the situation in other companies does not differ so greatly that the prevalence of affected farms in the population as a whole is much outside these confidence limits.

With regard to the relevance of the results to public health, the present survey detected FQ-resistant *E. coli* and *Campylobacter* in animal faeces from high proportions of farms although there were differences, both between farmed species and between bacterial species. There was a significantly lower level of resistance in *Campylobacter* amongst poultry compared with pigs, which may reflect a higher innate tendency of *C. coli*, the predominant pig species, to acquire resistance compared with *C. jejuni*, which predominates amongst poultry (Gupta *et al.* 2004; Boes *et al.* 2005). The prevalence of resistant *E. coli* was significantly higher than that of resistant *Campylobacter* on poultry farms, particularly for broilers. This may reflect seasonal and age-related variation in prevalence observed for *Campylobacter* and/or differences in carry-over of resistant *Campylobacter* spp. and *E. coli* between batches and in the farm environment following cleaning and disinfection.

The consumption of livestock food products is believed to be a risk factor for humans acquiring a FQ-resistant *Campylobacter* infection, and a recent red meat abattoir survey performed in Great Britain (Veterinary Laboratories Agency, unpublished data) suggested that 2% of *C. jejuni* and 11% of *C. coli* recovered from rectal or caecal contents of cattle, sheep and pigs were resistant to ciprofloxacin using the England and Wales Health Protection Agency breakpoint of 1 mg l$^{-1}$. However, the majority of FQ-resistant *Campylobacter* spp. were isolated from pigs, with no resistant isolates recovered from sheep. Results of genotyping and phenotyping suggest that pig strains have significantly lower overlap with human-derived campylobacters than do poultry-derived strains (Kramer *et al.* 2000; Guevremont *et al.* 2004; Siemer *et al.* 2005).

The situation in respect of poultry appears subject to significant geographical variation. The prevalence of FQ resistance is high in the Netherlands, with surveillance from 2004 indicating resistance in 40.4% of *C. jejuni* isolates (Anon 2005b). In France, the prevalence of resistance appears to have fallen in recent years (Gallay *et al.* 2007) and FQ resistance in *C. jejuni* from broilers in Scandinavia and Northern Ireland is generally around or below 5% of isolates (Oza *et al.* 2003; Bengtsson *et al.* 2005; Norstrom *et al.* 2006). Therefore, whilst in some areas poultry should be considered as a significant risk for human consumption of FQ-resistant *Campylobacter*, other important factors should not be overlooked. Indeed, foreign travel is consistently reported as the most important risk factor for human infections with an FQ-resistant *Campylobacter* in Scandinavia (Osterlund *et al.* 2003; Engberg *et al.* 2004; Norstrom *et al.* 2006). Another possible risk is acquisition from domestic pets (Moore *et al.* 2002).

The detection methods used in the present study were designed to maximise sensitivity, and a substantial proportion of farms were subsequently classified as ‘affected’. However, the proportion of animals that were excreting resistant bacteria on farm or at slaughter is unknown. The probability that consumers are exposed to FQ-resistant bacteria through contamination of carcases is highly dependent upon this latter proportion and upon further factors, including the fraction of the bacterial population that is resistant (for *E. coli* in the current study, often less than 1%), and the degree of gut spillage, cross-contamination and organism survival in the food processing and retail chain. Further epidemiological studies, linked to risk modelling, would be required to assess fully the potential risk to public health posed by FQ-resistant *E. coli* and *Campylobacter* of food animal origin.
Acknowledgements

This work was funded by the UK Veterinary Medicines Directorate and Department for Environment, Food and Rural Affairs, under project VM02101. The authors acknowledge the input of Dr A. Wilsmore and Dr J. Leslie at the inception of the project and thank Mr A. Cook and Mr A. Miller for collaboration on sampling of pig farms within Defra project OZ0316. They thank the veterinary surgeons, farmers and companies without whose support this project could not have been conducted and the laboratory staff at VLA who conducted the testing. Bayer kindly donated the ciprofloxacin used in the study.
### Table 1 Prevalence, with 95% confidence intervals ($CI_{95}$), of farms where fluoroquinolone-resistant *Campylobacter* spp. were detected

<table>
<thead>
<tr>
<th>Farm type</th>
<th>Total farms sampled</th>
<th>Farms with FQ-resistance</th>
<th>Number</th>
<th>%</th>
<th>Weighted estimate (%)</th>
<th>Lower $CI_{95^*}$ (%)</th>
<th>Upper $CI_{95^*}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler</td>
<td>68</td>
<td></td>
<td>12</td>
<td>17.6</td>
<td>19.4</td>
<td>11</td>
<td>31</td>
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<tr>
<td>Turkey</td>
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<td>8</td>
<td>38.1</td>
<td>41.5</td>
<td>22</td>
<td>66</td>
</tr>
<tr>
<td>All poultry</td>
<td>89</td>
<td></td>
<td>20</td>
<td>22.5</td>
<td>23.4</td>
<td>15</td>
<td>34</td>
</tr>
<tr>
<td>Pig</td>
<td>108</td>
<td></td>
<td>81</td>
<td>75.0</td>
<td>75.0</td>
<td>66</td>
<td>83</td>
</tr>
</tbody>
</table>

### Table 2 Prevalence, with 95% confidence intervals ($CI_{95}$), of farms where fluoroquinolone-resistant *E. coli* were detected

<table>
<thead>
<tr>
<th>Farm type</th>
<th>Total farms sampled</th>
<th>Farms with FQ-resistance</th>
<th>Number</th>
<th>%</th>
<th>Weighted estimate (%)</th>
<th>Lower $CI_{95^*}$ (%)</th>
<th>Upper $CI_{95^*}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<td>42</td>
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<tr>
<td>Pig</td>
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<td>63</td>
<td>58.3</td>
<td>58.3</td>
<td>48</td>
<td>68</td>
</tr>
</tbody>
</table>
Figure 1 Distribution of resistant organisms isolated from farms
Resistance patterns: □ no resistance; □ E. coli only resistant;
■ Campylobacter only resistant; ▣ both E. coli and Campylobacter resistant
Figure 2 Proportion of fluoroquinolone-resistant *E. coli* in 23 individual sample pools, each containing resistant *E. coli*, from eight poultry (■) and seven pig (■) farms.
REFERENCES


Anon (2005a) DANMAP 2004 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. Søborg: Danish Institute for Food and Veterinary Research.


