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Farm level risk factors for fluoroquinolone resistance in *E. coli* and thermophilic *Campylobacter* spp. on poultry farms

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Running head: Fluoroquinolone resistance in poultry

1 ## *Summary*

2 Data on husbandry practices, performance, disease and drug use were collected using

3 detailed questionnaires during a cross-sectional survey of 89 poultry meat farms in

4 England and Wales to provide information on possible risk factors for the occurrence

5 of fluoroquinolone (FQ) resistant bacteria on poultry meat farms. Faeces samples

6 taken as part of the surveys were used to classify farms as 'affected' or 'not affected'

7 by FQ-resistant *E. coli* or *Campylobacter* spp., and statistical analyses were

8 performed to identify factors associated with the farms' FQ resistance status.

9 The use of FQ on the farms was by far the most important factor influencing the

10 occurrence of FQ-resistant bacteria. Resistant *E. coli* and/or *Campylobacter* spp. were

11 found on 86% of the farms with a reported history of FQ use. However, resistant

12 bacteria were also found on a substantial proportion of farms with no history of FQ

13 use, suggesting that resistant organisms may spread between farms. Further analysis

14 suggested that there are differences in the importance of various factors between the

15 two organisms. For *Campylobacter* spp., on-farm hygiene, cleaning and disinfection

16 between batches of birds and wildlife control were of most significance. For *E. coli*,

17 biosecurity factors protecting the premises from outside contamination were of

18 particular importance, although the statistical modelling indicated that other factors

19 are likely to be involved. Detailed studies on a small number of poultry sites showed

20 that FQ-resistant *E. coli* can survive routine cleaning and disinfection, so this must be

21 of a high standard to reduce the persistence of resistant organisms on the farm.

22 It appears difficult to avoid the occurrence of resistant bacteria when FQ are used on a

23 farm, but the present findings provide evidence to support recommendations to reduce

24 the substantial risk of the incidental acquisition of such resistance by farms where FQ

25 are not used.

26

2

Introduction

Antimicrobial resistance amongst farm strains of enteric zoonotic bacteria, such as *E. coli* and thermotolerant *Campylobacter* spp., is of concern, particularly in view of the risk it presents for human disease, persistent enteric colonisation and (theoretically) transmission of resistance to other enteric bacteria (ECDC/EFSA/EMA, 2015). *E. coli* is a ubiquitous enteric commensal in both human and veterinary species, with a small subset of strains that present veterinary, human and cross-species disease hazards due to particular colonisation factors and/or toxins (Hartl and Dykhuizen, 1984). *Campylobacter* spp. are the most commonly identified human gastrointestinal pathogens reported in the European Union, confirmed in over 220000 cases in 2011 (EFSA/ECDC, 2013).

In recent community-wide data from the European Union resistance to the fluoroquinolone (FQ) antibiotic ciprofloxacin was found to be high (44% to 78% of isolates overall, depending on source and subspecies) among *Campylobacter jejuni* and *Campylobacter coli* isolates from human (mostly clinical) and broiler (monitoring) sources (EFSA/ECDC, 2014). A survey of 145 *Campylobacter* spp. isolates from human, milk, poultry and cattle sources in Italy similarly found 63% exhibiting ciprofloxacin resistance but comparatively little resistance to other tested antimicrobials, with the exception of tetracycline (Di Giannatale et al., 2014). A survey in Chile revealed a similarly high frequency of ciprofloxacin resistance among poultry and human *Campylobacter* spp. isolates (around 60%), whilst only 18% of isolates from cattle were resistant (Gonzalez-Hein et al., 2013). For *Campylobacter*, all these data are in the context of subtyping studies indicating that 50% to 80% of

51   human cases may be linked, directly or indirectly, to the chicken reservoir, and of FQ

52   being one of the principal drugs of choice for treating human campylobacteriosis

53   (Agunos et al., 2013; EFSA, 2010).

54

55   Aggregated European Community data for *E. coli* isolates from broilers showed,

56   similarly to *Campylobacter* spp., that over 50% of isolates were resistant to

57   ciprofloxacin (EFSA/ECDC, 2014). A sampling study provided evidence for the

58   dissemination of individual and multiple antimicrobial resistances in *E. coli* from

59   turkeys and broilers to their human handlers (van den Bogaard et al., 2001).

60   Furthermore, FQ-resistant isolates from human bacteraemias and faeces were found to

61   be more closely related to chicken isolates than to FQ-susceptible human isolates in

62   another study (Johnson et al., 2006).

63

64   Data from Australia, where FQ are restricted in the medical field and not used in food

65   animals, has shown that FQ resistance among human *Campylobacter* spp. isolates has

66   been slow to emerge, compared with other territories. Similarly, there is a low

67   frequency of FQ resistance among Australian human disease-causing *E. coli* isolates

68   (Cheng et al., 2012).

69

70   Attempts at restricting antimicrobial resistance on farms have included various

71   guidelines for the prudent use of veterinary antimicrobials (AAAP-AVMA, 2015;

72   OIE, 2014; RUMA, 2005). However, these have been based in large part upon expert

73   opinion, as published analyses of risk factors for the development of such resistances

74   are lacking.

75

76   The present report details a risk factor analysis performed following a survey for the

77   prevalence of FQ resistance among *E. coli* and *Campylobacter* spp. on poultry units in

78   the UK. Questionnaire data was used in conjunction with the prevalence results to

79   analyse FQ resistance with respect to a range of environmental and management

80   factors. The overall prevalence results for poultry and pigs and the analysis for risk

81   factors on pig farms have been reported elsewhere (Taylor et al., 2009, 2008)

82

83   ## *Materials and Methods*

84   ### Data collection

85   Two programmes of sampling were undertaken. For the first, 89 poultry meat farms

86   were included in a cross-sectional survey of FQ-resistant (FQr) *E. coli* and

87   *Campylobacter* spp., the details of which are described elsewhere (Taylor et al.,

88   2008). Briefly, 68 broiler and 21 turkey farms were each sampled once between June

89   2001 and June 2003, with 64 separate fresh floor droppings being collected from

90   random locations in up to four houses and combined into eight pools of eight samples

91   each. The sample size and sampling strategy were designed to give a 95% probability

92   of detecting resistant isolates if at least 5% of animals in the sampled houses were

93   shedding resistant bacteria and laboratory detection was 90% sensitive.

94

95   Sampling on poultry company premises was performed either by company-appointed

96   poultry veterinarians or by poultry company staff under the supervision of the

97   company veterinarian. Independent poultry producers (20 farms) carried out the

98   sampling themselves. To provide information on possible factors associated with

99   farms' FQ resistance status, data about husbandry practices, performance, disease and

100   drug use, including use of non-FQ antibiotics, were collected using detailed

101   questionnaires filled in by the farm manager with the veterinarian doing the sampling,

102   or by independent producers themselves. Data on antibiotic use was acquired, in the

103   large majority of cases and by all large units, by reference to detailed treatment

104   records in the farm diaries. These records are audited regularly for the purposes of

105   quality assurance and food chain protection.

106

107   The second (follow-up) programme investigated the potential for dissemination and

108   persistence of FQr organisms by carrying out farm-level sampling at representative

109   stages of breeding and production networks in two integrated companies. Faeces

110   sampling and data collection were carried out by the farm manager, according to the

111   protocols used for the first study, in five breeding flocks on repopulation, nine

112   breeding flocks in mid to late lay and 28 broiler flocks in mid to late rear. On a

113   selected proportion of sites where FQr organisms were found, intensive sampling was

114   performed by staff from the research team to investigate the distribution of resistant

115   *E. coli* on premises and to study their survival after cleaning and disinfection (C&D).

116   Samples taken on VLA sampling visits included faeces, water, dust and surface swabs

117   from building structures and equipment, as well as swabs from deep cracks in walls

118   and floors.

119

120   **Bacteriology**

121   Bacteriological analysis of faeces pools was performed using liquid media (buffered

122   peptone water [BPW] and Exeter's Enrichment Broth for *E. coli* and *Campylobacter*

123   spp., respectively) and selective solid media with added 1.0 mg/l ciprofloxacin

124   (Chromagar ECC for *E. coli*; sheep blood agar plus Skirrow's antibiotic supplement

125   and cefoperazone [BASAC] for *Campylobacter* spp.) as previously described (Taylor

126   et al., 2008). Farms were thus classified as 'affected' or 'not affected' with respect to

127   FQr *E. coli* or *Campylobacter* spp., using a selective concentration of ciprofloxacin

128   that is similar both to contemporaneous tentative breakpoints (Luber et al., 2003;

129   USDA, 2005), and the current European clinical breakpoint (EUCAST, 2014).

130   Putative *E. coli* colonies were confirmed using standard biochemical tests,

131   campylobacters were identified to species level by standard microbiological

132   procedures, and minimum inhibitory concentration (MIC) values of ciprofloxacin

133   were determined as described elsewhere (Taylor et al., 2008). Non-faeces samples

134   from intensive sampling visits in the second sampling programme were incubated in

135   approximately 10-fold volumes of BPW (225 ml for surface swabs) and incubated as

136   for faeces samples, before plating onto Chromagar ECC. Serotyping, toxin testing and

137   antibiograms (not including FQ) by the disc diffusion method were carried out using

138   standard protocols.

139

140   **Statistical analyses**

141   Statistical analyses were conducted using data from the first sampling programme

142   only. Associations between FQ use and farm types, and between FQ use and the

143   presence of FQr target organisms, were investigated using Chi-squared and Fisher's

144   exact tests. Calculations of relative risks associated with reported FQ use, with 95%

145   confidence intervals, were carried out using EpiInfo version 6 (Centers for Disease

146   Control and Prevention U.S.A. & World Health Organisation, Geneva, Switzerland).

147

148   Correlation and cluster analyses and logistic regression modelling were carried out

149   using SAS version 8 (SAS, 1999). The approach taken was exactly the same as that

150   used in analysing data from pig farms (Taylor et al., 2009). Briefly, the questionnaire

151   data were first placed in blocks according to subject matter (e.g. farm characteristics,

152   farm hygiene, biosecurity, drug usage including other antibiotics) and then the

153   variables within each block were screened using Ward's minimum variance cluster

154   analysis to identify groups of related variables (Everitt, 1980; Ward, 1963). From each

155   group thus identified, a representative variable was selected (using epidemiological

156   significance plus data variability and completeness as criteria) as a candidate

157   explanatory variable in logistic regression modelling *within* each block of variables,

158   with the presence on a farm of FQr *E. coli* or FQr *Campylobacter* spp. as outcome

159   variables.

160   By this method a number of candidate explanatory variables were identified from

161   each block. These variables were re-analysed by Ward's minimum variance cluster

162   analysis regardless of their block of origin. Some variables closely correlated with

163   other, more epidemiologically pertinent, ones were removed from the analysis at this

164   stage. The retained candidate variables from all blocks were then tried together in

165   logistic regression modelling, with results given as a list of risk factors for occurrence

166   of FQ resistance in each bacterial species, quantified in terms of adjusted odds ratios.

167   An r^2 value, that estimates the proportion of variation in the data explained by the

168   model, was calculated for each model, according to the method of Nagelkerke (1991)

169   as recommended by Collett (2003).

Results

Bacteriological findings

First sampling programme. Findings have been reported in detail by Taylor *et al.* (2008). FQr *E. coli* were isolated from 53 of the 89 farms. FQr *Campylobacter* spp. were isolated from 20 of the 89 farms. Of tested isolates obtained from the 1.0 mg/ml ciprofloxacin screening plates used, 79% of *E. coli* and 70% of *Campylobacter* spp. isolates had MIC values for ciprofloxacin of 16 mg/l or greater.

Second sampling programme. Of the five breeding flocks tested on repopulation in this follow-up investigation, none reported use of FQ during the previous six months or yielded FQr *E. coli* or *Campylobacter* spp.. Among the nine breeding flocks tested in mid- to late lay, FQr *E. coli* was isolated from two, but FQr *Campylobacter* spp. was not isolated. One of these nine flocks reported FQ use (in one of two houses) in the previous six months. Of the 28 broiler farms tested in mid-late rear, 25 yielded FQr *E. coli*. No FQr *Campylobacter* spp. was isolated. FQ had been administered during the previous six months on only one of these farms, in non-sampled parts of the farm, and all samples from this farm yielded FQr *E. coli*.

Further intensive sampling visits, for FQr *E. coli,* were carried out at one of the mid-lay breeding flocks, a linked company hatchery and after C&D on two of the commercial broiler sites. From the breeding flock, FQr *E. coli* was isolated from 16 of 100 environmental samples. It was most frequently found in fresh faeces and litter (rather than nest boxes), but also found in guttering and on the concrete apron outside the house. At the hatchery, FQr *E. coli* was found in six of the 100 samples taken from meconium and egg/chick waste, as well as on cleaned and disinfected surfaces.

195   On both post-C&D broiler farms, FQr *E. coli* was found in cracks and crevices,

196   pooled wash water, ante-rooms which had been less well disinfected and fresh

197   droppings from wild birds collected from the house exterior.

198

199   Seventy two *E. coli* strains from the second sampling programme were examined for

200   MIC and serotype. Isolates were from breeder units in mid-lay, broiler units and the

201   hatchery. Ciprofloxacin MIC values were $\geq$ 8 mg/l, with a modal value of 16 mg/l.

202   Eight serovars were identified, and 12 isolates proved untypable. There was no

203   overlap between identified serovars isolated from breeder versus broiler flocks. Thus,

204   there was no evident relationship between breeder and broiler isolates. One of the

205   three serovars isolated from the hatchery was associated with the breeder flocks, and

206   another with the broiler flocks.

207

208   From one company, *E. coli* O101:K+ (verocytotoxin-negative, MIC 32 mg/l) was

209   isolated in five broiler flocks in mid-late rear on one farm.. The same serovar was also

210   found on two other farms from the same company, in two sequential flocks on each

211   farm. FQr *E. coli* O9:K+ was isolated from two of the breeding flocks (MIC 8 mg/l)

212   and from a waste skip at the hatchery (MIC 16 mg/l). However, this serovar was not

213   amongst the isolates tested from broiler units within the company.

214

215   The 72 serotyped *E. coli* isolates were also tested for resistance to antibiotics. Several

216   patterns were found, with resistance to ampicillin (86% isolates),

217   sulphamethoxazole/trimethoprim (65% isolates), tetracycline (67% isolates) and

218   streptomycin (43% isolates) being the most frequently encountered, in addition to

219   quinolone/FQ resistance.

Use of antibiotics and risk of fluoroquinolone resistance on the

surveyed farms

The questionnaire response options in relation to use of FQ on farms were: 'within 12

months', 'between one and two years ago', 'over two years ago' and 'never'. The

responses are summarised in Table 1. Use of FQ was reported on 22 of 88 (25%)

poultry farms in the survey, with one no-response. FQ use was significantly (Chi^2

$p < 0.0001$) more common on turkey farms (14/21) than on broiler farms (8/67).

Among the broiler farms, FQ use was significantly (Chi^2 $p < 0.0001$) more common

by independent producers (7/18) than by large poultry company farms (1/49).

Amongst turkey farms the most recent use had been within a year on nine of the 14

farms that reported use. On broiler farms, only two of the eight reporting use of FQ

had done so within the last year (Table 1). On all except one farm, FQ were

administered through water medication. In turkeys, the most common problem treated

with FQ was reported as being '*E. coli* septicaemia'. Amongst broilers, the most

common problems reportedly associated with FQ use were 'yolk sac infections' or

'stunted chicks'. Use, in the previous 12 months, of non-FQ antibiotics other than

amoxicillin (41% of farms), lincospectin (22% of farms) and tetracycline (10% of

farms) was uncommon. Just under one fifth of farms reported routine use of in-feed

antibiotic.

FQr *E. coli* or FQr *Campylobacter* spp. were detected on 19 (86%) of the 22 farms

that had used FQ and 40 (61%) of the 66 farms that reported never using FQ. The

prevalence of farms positive for FQr *E. coli* or FQr *Campylobacter* spp. was not

significantly different between farms with most recent use of FQ over one year ago,

245 compared with those using FQ within the last year. Therefore, farms where any FQ

246 use was reported were grouped together for comparison with those farms reporting

247 that they had never used FQ in further analyses. Table 2 shows the relative risks (with

248 95% confidence intervals) for the occurrence of FQ resistance on poultry farms,

249 associated with the use of FQ.

250

251 Overall within-farm prevalence values for FQr *Campylobacter* spp. and *E. coli* were

252 around 5% and 20% of faecal pools, respectively. On some premises, resistant

253 *Campylobacter* were shed by birds in only one or two houses, but there were others

254 where shedding birds were present across the farm. Birds shedding FQr *E. coli* tended

255 to be distributed throughout the houses on affected farms.

256

257 **Modelling of risk factors for the occurrence of FQ-resistance**

258 Correlation and clustering analysis revealed that farm type (turkey or broiler;

259 independent grower or large company) was strongly correlated with several of the

260 variables. Specifically:

261 • Turkey farms were strongly *positively* correlated with the use of FQ, cleaning

262 and disinfecting header tanks, seeing more than five rats at depopulation, the use

263 of plastic drinkers for chicks, and the use of growth promoters and tetracyclines.

264 • Turkey farms were strongly *negatively* correlated with single-handed operation,

265 enclosure by a perimeter fence, the provision of wheel dips, wild bird access to

266 poultry houses, the presence of dogs or cats, cleaning and disinfecting ante

267 rooms, feed hoppers and areas outside houses, and the use of nipple drinkers and

268 digestive enzymes.

269   • Independent farms were strongly *positively* correlated with the use of FQ, the

270   presence of dogs or cats, slaughtering birds at an older age and cleaning and

271   disinfecting ante rooms.

272   • Independent farms were strongly *negatively* correlated with the provision of

273   masks and wheel dips, seeing more than five rats at depopulation, cleaning and

274   disinfecting header tanks, and the use of digestive enzymes and growth

275   promoters.

276

277   In addition, the correlation analysis indicated the following:

278   • Single-handed farms tended not to have wheel dips.

279   • Farms enclosed by a perimeter fence tended to provide wheel dips and have

280   dogs or cats.

281   • Farms enclosed by a perimeter fence tended not to have big houses, tended not

282   to be turkey farms and, therefore, tended not to use growth promoters and

283   tetracycline.

284   • Larger farms tended to provide masks to staff.

285   • Dusting of all detailed areas was positively correlated with wet cleaning of all

286   detailed areas and removal of all wash water from the site.

287   • C&D of ante rooms was strongly positively correlated with C&D of feed

288   hoppers.

289   • In this particular sample of poultry farms, the variable 'provision of a mask' was

290   also positively correlated with provision of hat and gloves and provision of hand

291   sanitiser and provision of a toilet.

292

293 The turkey farm type was very strongly associated with the use of FQ. The turkey

294 farm variable itself was not significant in the final models. This implies that the

295 reason for the increased proportion of turkey farms with FQr *E. coli* or

296 *Campylobacter* spp., compared with broiler farms, as reported previously (Taylor et

297 al., 2008), is fully explained by other variables in the model, chiefly the use of FQ on

298 the farms.

299

300 The results of the final regression modelling are presented in tables 3 and 4 showing

301 the variables included as risk factors, estimates of coefficients with p-values, the

302 estimated adjusted odds ratios with 90% and 95% confidence intervals and the r^2

303 value.

304

305 Having fitted main effects, several interactions were identified as statistically

306 significant but inclusion of these in the regression models always resulted in estimates

307 for some odds ratios approaching infinity or zero. This was considered to be the result

308 of small sample sizes, such that inclusion of too many effects, notably the

309 interactions, produced models that were 'over-fitted', as described by Collett (2003).

310 To avoid the possibility of over-fitting and implausible interpretations, models were

311 finalised without interactions.

312

313 Table 3 provides a summary of the factors included in the final fitted logistic

314 regression model for the risk of occurrence of FQr *E. coli*. Significant factors

315 increasing risk are: use of FQ in past, single-handed operation of the site, and the

316 existence of a public footpath on the periphery of the site. The sole significant factor

317 decreasing risk is enclosure of the site by a perimeter fence. The r^2 value of the fitted

318   model is fairly low, which indicates that other, unidentified, explanatory risk factors

319   are likely to be involved.

320

321   Table 4 provides a summary of the factors included in the final fitted logistic

322   regression model for the risk of occurrence of FQr *Campylobacter* spp. Significant

323   risk factors increasing risk are: the use of FQ in the past and wild birds having access

324   to poultry houses. Significant factors decreasing risk are: more than the median (for

325   all broiler or turkey farms in the sample, as appropriate) number of birds on site, the

326   site operated by an independent grower, masks provided for staff, detailed areas

327   dusted before wet cleaning, and feed hoppers cleaned and disinfected.

328

329   The r^2 value is over 50%, indicating that the model provides a good explanation of

330   factors affecting the occurrence of FQ-resistant *Campylobacter* spp.. However, the

331   model is fitted with quite a large number of variables (seven) in relation to the dataset

332   size (n = 84) and is in danger of being 'over-fitted'. The result of this is the relatively

333   wide confidence intervals for the adjusted odds ratios. Nevertheless, the fitted

334   variables are statistically significant. The conclusion is that the factors in the model

335   affect risk significantly, and perhaps greatly, but the data are not sufficient to allow

336   the risk effect to be quantified very precisely.

337

338   ### *Discussion*

339   The bacteriological findings of the initial survey (Taylor et al., 2008) and the follow-

340   up studies reported here have identified the frequent occurrence of *E. coli* and

341   *Campylobacter* spp. with FQ resistance on a substantial proportion of turkey and

342 broiler commercial production facilities. FQr *E. coli* were also isolated on breeding

343 flock premises. Moreover, the FQr *E. coli* and *Campylobacter* spp. typically exhibited

344 clinically-significant elevations in MIC values (Becnel Boyd et al., 2009; EUCAST,

345 2014) and the FQr *E. coli* often showed resistance to other classes of antimicrobial

346 agents. The present findings for *E. coli* are similar, in terms of frequency of isolation

347 on FQ resistance-selective media, MIC values observed, and common co-resistances

348 with other classes of antimicrobial drugs, with the findings of Gosling *et al*. (2012).

349 That study used UK-wide samples from turkey units taken for a European Union

350 baseline survey.

351

352 It was initially hypothesised that FQr organisms would be found on a small

353 percentage of farms, principally those where FQ were used. However, in the first

354 (structured) survey FQr organisms (mostly *E. coli*) were detected on a heavy majority

355 (86%) of farms that had used FQ in the past, and also on over half (61%) of the farms

356 that reported never using FQ. This finding is similar to that of a concurrent survey in

357 pig production (Taylor et al., 2009). A history of FQ use was associated with an

358 approximately doubled risk that FQr *E. coli* or *Campylobacter* spp. would be found on

359 a farm, and with the highest odds ratios among all the factors considered in the

360 logistic regression models for FQ resistance on farm.

361

362 The substantial prevalence of FQ resistance-affected farms that had never used FQ

363 suggests that FQr organisms may commonly be imported onto farms, either with

364 replacement birds in the case of *E.coli*, or from environmental sources in the case of

365 *Campylobacter* spp.. The persistence of such strains correlates with experimental data

366 suggesting little or no fitness cost associated with a moderate degree of FQ resistance

367   in *E. coli* (Schrag et al., 1997) and *Campylobacter* spp. (Q. Zhang et al., 2003). This is

368   consistent with the experience in countries where FQ are either prohibited or not

369   specifically licensed in poultry farming (USA, Canada and Denmark), where FQ

370   resistance among *Campylobacter* spp. isolates from poultry sources has not

371   consistently declined following cessation of FQ use in the sector (Agunos et al., 2013;

372   DANMAP, 2014).

373

374   There are, inevitably, some reasons to be careful in interpreting the present analysis.

375   The influence of co-resistance involving FQ resistance plus other antibiotics needs

376   some consideration, despite no significant associations being found between FQ

377   resistance on premises and recent use of a specific antibiotic class.

378

379   In *Campylobacter* spp., resistance to FQ typically is mediated by mutation of a

380   chromosomally-encoded topoisomerase, which is a mechanism specific to quinolone

381   antibiotics (Gyles, 2008; Qijing Zhang et al., 2003). This is augmented in some cases

382   by overexpression of the chromosomally-encoded multi-drug efflux pump CmeB

383   (Fàbrega et al., 2008). Therefore, clinical resistance to FQ is unlikely to occur

384   consequent upon use of a different antibiotic class or by introduction on mobile

385   genetic elements. However, as shown in the present study and elsewhere (Pérez-Boto

386   et al., 2013), FQ resistance in *Campylobacter* spp. from poultry farms is often

387   accompanied by other antibiotic resistances in the same isolates. If FQ resistance is,

388   for whatever reason, more common amongst antibiotic-resistant strains than among

389   susceptible strains, then co-selection by other antibiotics may maintain pre-existing

390   FQr strains for a prolonged period, especially if , as appears to be the case, the fitness

391   cost of FQ resistance among *Campylobacter* spp. is low (Luo et al., 2005). It is

392   therefore important to note that, whereas FQ resistance clearly has the potential to

393   persist in the absence of FQ use by co-selection, it seems unlikely to be present in the

394   first instance without either being introduced from elsewhere, or following selection

395   by FQ use.

396

397   For *E. coli*, the picture is perhaps more complicated. High-level FQ resistance is

398   firmly associated with topoisomerase mutation(s) (Fàbrega et al., 2008; Gyles, 2008;

399   Vanni et al., 2014), although intermediate resistance or enhancement of clinical

400   resistance is possible by chromosomal efflux pump upregulation and/or plasmid-borne

401   genes encoding target site protection (*qnr*), efflux (*qepA*) or FQ modification by an

402   aminoglycoside acetyltransferase (*aac(6′)-Ib-cr*) (Fàbrega et al., 2008; Veldman et

403   al., 2011; Yue et al., 2008). Therefore, intermediate FQ susceptibility may be

404   introduced or maintained by horizontal transfer and/or co-selection by the use of other

405   antibiotic classes. However, no non-FQ antibiotics are likely to select the spontaneous

406   topoisomerase mutations fundamental to clinical resistance levels.

407

408   Although the prevalence of FQ resistance among contemporaneous diagnostic avian

409   samples of *E. coli* in the UK was low (around 2% to 6% depending on region and

410   source), resistances to commonly-used antimicrobials were more prevalent, in the

411   range 23% to 65% of isolates for ampicillin, amoxicillin, spectinomycin and

412   trimethoprim/ sulphonamide (Anon., 2007), consistent with the resistance findings in

413   the present study. This suggests that many FQ-resistant *E. coli* would also have had

414   resistance to other therapeutic antibiotics. Like *Campylobacter* spp., this might

415   facilitate co-selection of FQ resistance by other antibiotics but would not be expected

416   to generate *de novo* the clinical degree of resistance seen in the present study.

417 The second sampling programme and typing studies reinforce the finding of the initial

418 survey that the presence of FQr *E. coli* on a farm may not necessarily be related to

419 recent recorded use of FQ on the premises. The FQr *E. coli* isolated belonged to

420 numerous serogroups and had a range of different antibiograms, indicating that they

421 did not belong to a single clone. Furthermore, the FQr *E. coli* on the two farms tested

422 after C&D were able to persist in the environment and were a potential source of

423 infection for a new flock. A pertinent allied observation from the initial survey is that,

424 on farms where FQ had been used, there was no significant effect seen of the time

425 elapsed since last use upon the risk of FQ resistance. It is interesting to note in this

426 context that Ingram *et al.* (2013) isolated FQr *E. coli* harbouring multidrug-resistance

427 plasmids from chicken carcasses in Australia (a territory where FQ are not licensed

428 for poultry), thereby showing that topoisomerase-mutants may be present commonly

429 in products from apparently FQ-free systems.

430

431 The second sampling study also provided observational evidence that, for *E. coli* at

432 least, FQr strains potentially can transfer between broiler premises within integrated

433 operations, presumably via personnel and fomites. There was no evidence of vertical

434 transmission of FQr *E. coli* from breeder to broiler flocks, which may reflect the

435 biosecurity barrier that can be achieved between these levels of production by

436 hygienic hatchery management.

437

438 The differences in risk factors identified for the two bacterial genera examined may

439 reflect differences in the usual modes of transfer of these organisms between

440 locations. Interested readers are directed to Taylor *et al.* (2009) for discussion of the

441 merits and limitations of the statistical modelling approach of the present study. In

442   addition to FQ use and single-handed operation, the two variables identified as

443   significant risk factors for the occurrence of FQr *E. coli* were the existence of a

444   perimeter fence (protective) and of a public footpath (increasing risk). Thus, in

445   common with pig units, biosecurity appears to be of high importance for FQr *E. coli*.

446   For poultry the physical integrity of the farm limits seems to be of primary

447   significance, whereas for pigs the proximity of other pig units and visitor biosecurity

448   was found to be important (Taylor et al., 2009).

449

450   These differences in the most significant biosecurity barriers for pigs versus poultry

451   farms may to some extent reflect differences in the frequency of visitors and of feed

452   and stock transporters, differences in the housing systems, in the typical farm sizes,

453   and in the typical local environments. Whilst risk factor analysis may identify areas of

454   particular vulnerability or strength for particular enterprise types, examination of any

455   particular unit would sensibly include a comprehensive overview of biosecurity

456   issues, especially as the relatively low r^2 value for the *E. coli* model indicates other

457   significant unidentified risk factors that may not be common to all or most units.

458

459   For *Campylobacter* spp., the risk factor model for the occurrence of FQ resistance

460   indicates the importance of farm hygiene, perhaps reflecting the greater importance of

461   shorter-range transmission between animals for this more environmentally labile

462   pathogen when compared with *E. coli*. One protective factor of particular interest was

463   provision of a mask. This factor was positively correlated with, and effectively a

464   proxy variable for, other factors including the provision of hand sanitisers, a toilet,

465   hats and gloves. The inclusion of this factor in the model can be taken as indication of

466   the protective effect of better hygiene facilities in general.

467

468   The significantly protective variables regarding dusting (of several difficult or

469   inaccessible parts of poultry houses before wet cleaning) and C&D of feed hoppers

470   are interpreted as indicators of generally superior farm cleaning. Campylobacters are

471   frequently recovered from puddles and other wet locations on farms, but typically not

472   from dry materials. The findings indicate the importance of attention to detail when

473   cleaning between crops, presumably by preventing carry-over of infection,

474   particularly of *Campylobacter* spp., between batches of stock.

475

476   The introduction of *Campylobacter* spp. (including, potentially, FQr strains) to a

477   poultry flock or premises is considered to be a more important issue than carry-over,

478   and may occur following the repeated entrance of staff with contaminated clothing,

479   hands or equipment (Newell et al., 2011). The risks of acquisition of *Campylobacter*

480   spp. by flocks before slaughter are related to several factors including: season, on-

481   farm hygiene, other animal species on the farm, more than one poultry house per

482   stockperson, thinning of slaughter-age flocks by catching crews and features of the

483   farm environmental surroundings, as reviewed by Vidal *et al.*, (2014). However,

484   Refregier-Petton *et al.* (2001) reported a risk factor analysis for the presence of

485   *Campylobacter* spp. in broilers at slaughter using a similar methodology to the present

486   one and found, amongst other things, that no specific stockperson hygiene practices

487   were significant. Discrepancies noted in that report between claimed and observed

488   hygiene practices may explain this finding, and its apparent lack of concordance with

489   the present evidence.

490

491   The transmission of FQr *Campylobacter* spp., and probably of *Campylobacter* spp.

492   more generally, may also be associated with wildlife vectors. Remarkable suppression

493   of seasonal peaks in flock *Campylobacter* spp. colonisation has been demonstrated, in

494   the context of good general hygiene, following the use of mesh screens to exclude

495   wildlife down to the level of flying insects from broiler houses (Bahrndorff et al.,

496   2013). The factor, 'saw more than five rats at last depopulation' was associated with

497   an increased risk, but was not significant in the final model. Access to the poultry

498   houses by wild birds was a significant factor for increasing risk in the final model,

499   with a large odds ratio. It has been documented that wild birds carry *Campylobacter*

500   spp., including FQr strains (Broman et al., 2002; Waldenstrom et al., 2005), although

501   wild bird strains generally differ from poultry and human strains (Broman et al.,

502   2004). Access by wild birds may be indicative of poorer biosecurity with respect to

503   wildlife more generally.

504

505   In conclusion, the present investigations have illustrated the strong association

506   between any use of FQ on poultry farms and the presence of *E. coli* and/or

507   *Campylobacter* spp. with clinically-relevant levels of resistance to FQ on the same

508   premises. Furthermore, the introduction or maintenance of FQr organisms on farms

509   appears significantly influenced by farm hygiene (*Campylobacter* spp.) and boundary

510   biosecurity (*E. coli*), with evidence also being found of cross-transfer of FQr *E. coli*

511   between premises linked in the production system. As has been discussed elsewhere

512   (Taylor et al., 2008), both *E. coli* and *Campylobacter* spp. are zoonotic organisms for

513   which FQ are therapeutic agents in humans. It appears, on the present evidence, to be

514   difficult for farms that use FQ to avoid the development of FQ-resistant *E. coli* and

515   *Campylobacter* spp. on farm. However, for those farms that do not use FQ, an

516   emphasis on excellence in biosecurity and on-farm hygiene is likely to prove

517   protective. The benefits of such a strategy are likely to extend to control or exclusion

518   of some other infectious agents also. This is in line with guidelines produced by the

519   UK 'Responsible Use of Medicines in Agriculture Alliance' (RUMA;

520   http://www.ruma.org.uk), which stress that the use of antimicrobials should be seen as

521   complementing good management, vaccination and site hygiene.

522

523

529

530   **Conflicts of interest**

531   None
532

References

AAAP-AVMA, 2015. American Association of Avian Pathologists - American Veterinary Medical Association guidelines for judicious therapeutic use of antimicrobials in poultry.

Agunos, A., Léger, D., Avery, B.P., Parmley, E.J., Deckert, A., Carson, C.A., Dutil, L., 2013. Ciprofloxacin-resistant *Campylobacter* spp. in retail chicken, western Canada. Emerg. Infect. Dis. 19, 1121–1124. doi:10.3201/eid1907.111417

Anon., 2007. Overview of antimicrobial usage and bacterial resistance in selected human and animal pathogens in the UK: 2004. Communicable Disease Surveillance Centre Northern Ireland, Department for Environment Food and Rural Affairs, Department of Agriculture and Rural Development Northern Ireland, Department of Health, Food Standards Agency, Health Protection Agency, Health Protection Scotland, Scottish Agricultural College, Scottish Executive Environment and Rural Affairs Department, Veterinary Laboratories Agency, Veterinary Medicines Directorate.

Bahrndorff, S., Rangstrup-Christensen, L., Nordentoft, S., Hald, B., 2013. Foodborne disease prevention and broiler chickens with reduced *Campylobacter* infection. Emerg. Infect. Dis. 19, 425–430. doi:10.3201/eid1903.111593

Becnel Boyd, L., Maynard, M.J., Morgan-Linnell, S.K., Horton, L.B., Sucgang, R., Hamill, R.J., Jimenez, J.R., Versalovic, J., Steffen, D., Zechiedrich, L., 2009. Relationships among ciprofloxacin, gatifloxacin, levofloxacin, and norfloxacin MICs for fluoroquinolone-resistant *Escherichia coli* clinical isolates. Antimicrob. Agents Chemother. 53, 229–234. doi:10.1128/AAC.00722-08

Broman, T., Palmgren, H., Bergstrom, S., Sellin, M., Waldenstrom, J., Danielsson-Tham, M.L., Olsen, B., 2002. *Campylobacter jejuni* in black-headed gulls (Larus ridibundus): Prevalence, genotypes, and influence on C. *jejuni* epidemiology. J. Clin. Microbiol. 40, 4594–4602.

Broman, T., Waldenstrom, J., Dahlgren, D., Carlsson, I., Eliasson, I., Olsen, B., 2004. Diversities and similarities in PFGE profiles of *Campylobacter jejuni* isolated from migrating birds and humans. J. Appl. Microbiol. 96, 834–843.

Cheng, A.C., Turnidge, J., Collignon, P., Looke, D., Barton, M., Gottlieb, T., 2012. Control of fluoroquinolone resistance through successful regulation, Australia. Emerg. Infect. Dis. 18, 1453–1460. doi:10.3201/eid1809.111515

Collett, D., 2003. Models for binary and binomial data, in: Modelling Binary Data. Chapman & Hall/CRC, London, pp. 45–102.

DANMAP, 2014. DANMAP 2013 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Danish Integrated Antimicrobial Resistance Monitoring and Research Programme.

Di Giannatale, E., Di Serafino, G., Zilli, K., Alessiani, A., Sacchini, L., Garofolo, G., Aprea, G., Marotta, F., 2014. Characterization of antimicrobial resistance patterns and detection of virulence genes in *Campylobacter* isolates in Italy. Sensors 14, 3308–3322. doi:10.3390/s140203308

ECDC/EFSA/EMA, 2015. ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. EFSA J. 13, 4006; 114 pp.

581 EFSA, 2010. Scientific Opinion on quantification of the risk posed by broiler meat to
582   human campylobacteriosis in the EU. EFSA J. 8, 1437 [89 pp.].
583   doi:10.2903/j.efsa.2010.1437
584 EFSA/ECDC, 2014. The European Union Summary Report on antimicrobial
585   resistance in zoonotic and indicator bacteria from humans, animals and food in
586   2012. EFSA J. 12, 3590 (336 pp.). doi:10.2903/j.efsa.2014.3590
587 EFSA/ECDC, 2013. The European Union summary report on trends and sources of
588   zoonoses, zoonotic agents and food-borne outbreaks in 2011.
589 EUCAST, 2014. Breakpoint tables for interpretation of MICs and zone diameters.
590   Version 4.0.
591 Everitt, B.S., 1980. Cluster analysis, 2nd ed. Heinemann Educational Books Ltd.,
592   London.
593 Fàbrega, A., Sánchez-Céspedes, J., Soto, S., Vila, J., 2008. Quinolone resistance in
594   the food chain. Int. J. Antimicrob. Agents 31, 307–315.
595   doi:10.1016/j.ijantimicag.2007.12.010
596 Gonzalez-Hein, G., Cordero, N., Garcia, P., Figueroa, G., 2013. Molecular analysis of
597   fluoroquinolones and macrolides resistance in *Campylobacter jejuni* isolates
598   from humans, bovine and chicken meat. Rev. Chil. Infectologia 30, 135–139.
599 Gosling, R.J., Clouting, C.S., Randall, L.P., Horton, R.A., Davies, R.H., 2012.
600   Ciprofloxacin resistance in *E. coli* isolated from turkeys in Great Britain.
601   Avian Pathol. 41, 83–89. doi:10.1080/03079457.2011.640659
602 Gyles, C.L., 2008. Antimicrobial resistance in selected bacteria from poultry. Anim.
603   Health Res. Rev. 9, 149–158. doi:10.1017/S1466252308001552
604 Hartl, D.L., Dykhuizen, D.E., 1984. The population genetics of *Escherichia coli*.
605   Annu Rev Genet 18, 31–68.
606 Ingram, P.R., Rogers, B.A., Sidjabat, H.E., Gibson, J.S., Inglis, T.J.J., 2013. Co-
607   selection may explain high rates of ciprofloxacin non-susceptible *Escherichia*
608   *coli* from retail poultry reared without prior fluoroquinolone exposure. J. Med.
609   Microbiol. 62, 1743–1746. doi:10.1099/jmm.0.062729-0
610 Johnson, J.R., Kuskowski, M.A., Menard, M., Gajewski, A., Xercavins, M., Garau, J.,
611   2006. Similarity between human and chicken *Escherichia coli* isolates in
612   relation to ciprofloxacin resistance status. J Infect Dis 194, 71–8.
613 Luber, P., Bartelt, E., Genschow, E., Wagner, J., Hahn, H., 2003. Comparison of broth
614   microdilution, E test, and agar dilution methods for antibiotic susceptibility
615   testing of *Campylobacter jejuni* and *Campylobacter coli*. J. Clin. Microbiol.
616   41, 1062–1068. doi:10.1128/JCM.41.3.1062-1068.2003
617 Luo, N., Pereira, S., Sahin, O., Lin, J., Huang, S., Michel, L., Zhang, Q., 2005.
618   Enhanced *in vivo* fitness of fluoroquinolone-resistant *Campylobacter jejuni* in
619   the absence of antibiotic selection pressure. Proc. Natl. Acad. Sci. U. S. A.
620   102, 541–546. doi:10.1073/pnas.0408966102
621 Nagelkerke, N.J.D., 1991. A note on a general definition of the coefficient of
622   determination. Biometrika 78, 691–692.
623 Newell, D.G., Elvers, K.T., Dopfer, D., Hansson, I., Jones, P., James, S., Gittins, J.,
624   Stern, N.J., Davies, R., Connerton, I., Pearson, D., Salvat, G., Allen, V.M.,
625   2011. Biosecurity-based interventions and strategies to reduce *Campylobacter*
626   spp. on poultry farms. Appl. Environ. Microbiol. 77, 8605–8614.
627   doi:10.1128/AEM.01090-10
628 OIE, 2014. Chapter 6.9. Responsible and prudent use of antimicrobial agents in
629   veterinary medicine, in: Terrestrial Animal Health Code 2014. World
630   Organisation for Animal Health (OIE).

631   Pérez-Boto, D., García-Peña, F.J., Abad-Moreno, J.C., Echeita, M.A., 2013.
632   Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter*
633   *coli* strains isolated from two early stages of poultry production. Microb. Drug
634   Resist. 19, 323–330. doi:10.1089/mdr.2012.0160
635   Refregier-Petton, J., Rose, N., Denis, M., Salvat, G., 2001. Risk factors for
636   *Campylobacter* spp. contamination in French broiler-chicken flocks at the end
637   of the rearing period. Prev. Vet. Med. 50, 89–100.
638   RUMA, 2005. Responsible use of antimicrobials in poultry production.
639   Schrag, S.J., Perrot, V., Levin, B.R., 1997. Adaptation to the fitness cost of antibiotic
640   resistance in *Escherichia coli*. Proc. R. Soc. B Biol. Sci. 264, 1287–1291.
641   Taylor, N., Clifton-Hadley, F., Wales, A., Ridley, A., Davies, R., 2009. Farm level
642   risk factors for fluoroquinolone resistance in *E. coli* and thermophilic
643   *Campylobacter* spp. on finisher pig farms. Epidemiol. Infect. 137, 1121–1134.
644   doi:dx.doi.org/10.1017/S0950268808001854
645   Taylor, N.M., Davies, R.H., Ridley, A., Clouting, C., Wales, A.D., Clifton-Hadley,
646   F.A., 2008. A survey of fluoroquinolone resistance in *E. coli* and thermophilic
647   *Campylobacter* spp. on poultry and pig farms in Great Britain. J. Appl.
648   Microbiol. 105, 1421–31. doi:10.1111/j.1365-2672.2008.03877.x
649   USDA, 2005. National Antimicrobial Resistance Monitoring System (NARMS):
650   Enteric Bacteria. Veterinary Isolates, final report 2005. U.S. Department of
651   Agriculture, Agricultural Research Service, Athens, Georgia, USA.
652   van den Bogaard, A.E., London, N., Driessen, C., Stobberingh, E.E., 2001. Antibiotic
653   resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry
654   slaughterers. J. Antimicrob. Chemother. 47, 763–771.
655   Vanni, M., Meucci, V., Tognetti, R., Cagnardi, P., Montesissa, C., Piccirillo, A.,
656   Rossi, A.M., Bello, D.D., Intorre, L., 2014. Fluoroquinolone resistance and
657   molecular characterization of *gyrA* and *parC* quinolone resistance-determining
658   regions in *Escherichia coli* isolated from poultry. Poult. Sci. 93, 856–863.
659   doi:10.3382/ps.2013-03627
660   Veldman, K., Cavaco, L.M., Mevius, D., Battisti, A., Franco, A., Botteldoorn, N.,
661   Bruneau, M., Perrin-Guyomard, A., Cerny, T., De Frutos Escobar, C., Guerra,
662   B., Schroeter, A., Gutierrez, M., Hopkins, K., Myllyniemi, A.-L., Sunde, M.,
663   Wasyl, D., Aarestrup, F.M., 2011. International collaborative study on the
664   occurrence of plasmid-mediated quinolone resistance in *Salmonella enterica*
665   and *Escherichia coli* isolated from animals, humans, food and the environment
666   in 13 European countries. J. Antimicrob. Chemother. 66, 1278–1286.
667   doi:10.1093/jac/dkr084
668   Vidal, A.B., Davies, R.H., Rodgers, J.D., Ridley, A., Clifton-Hadley, F., 2014.
669   Epidemiology and control of *Campylobacter* in modern broiler production, in:
670   Sheppard, S.K., Méric, G. (Eds.), *Campylobacter* Ecology and Evolution.
671   Caister Academic Press, p. 360.
672   Waldenstrom, J., Mevius, D., Veldman, K., Broman, T., Hasselquist, D., Olsen, B.,
673   2005. Antimicrobial resistance profiles of *Campylobacter jejuni* isolates from
674   wild birds in Sweden. Appl. Environ. Microbiol. 71, 2438–2441.
675   Ward, J.H., 1963. Hierarchical grouping to optimise an objective function. J. Am.
676   Stat. Assoc. 58, 236–244.
677   Yue, L., Jiang, H.-X., Liao, X.-P., Liu, J.-H., Li, S.-J., Chen, X.-Y., Chen, C.-X., Lü,
678   D.-H., Liu, Y.-H., 2008. Prevalence of plasmid-mediated quinolone resistance
679   *qnr* genes in poultry and swine clinical isolates of *Escherichia coli*. Vet.
680   Microbiol. 132, 414–420. doi:10.1016/j.vetmic.2008.05.009

681   Zhang, Q., Lin, J., Pereira, S., 2003. Fluoroquinolone-resistant *Campylobacter* in
682   animal reservoirs: dynamics of development, resistance mechanisms and
683   ecological fitness. Anim Health Res Rev 4, 63–71.
684   Zhang, Q., Lin, J., Pereira, S., 2003. Fluoroquinolone-resistant *Campylobacter* in
685   animal reservoirs: dynamics of development, resistance mechanisms and
686   ecological fitness. Anim. Health Res. Rev. 4, 63–72.
687   doi:10.1079/AHRR200356
688

Table 1: Detection of fluoroquinolone (FQ)-resistant bacteria on poultry farms, compared with reported use of FQ

Last use of FQ antibiotics	Broiler farms	Turkey farms	All farms	Number with FQ resistance	
				E. coli	*Campylobacter*
In last year	2	9	11	10 (91%)	4 (36%)
Over 1 year ago	6[a]	5[b]	11	9 (82%)	4 (36%)
Never used	59	7	66	33 (50%)	11 (17%)

a: 2 of 6 reported most recent use over 2 years ago
b: 1 of 5 reported most recent use over 2 years ago

Table 2: Relative risks (with 95% confidence intervals) for the occurrence of fluoroquinolone (FQ) resistance on poultry farms, associated with the reported use of FQ

	Proportion of farms with FQ resistance	
	E. coli	*Campylobacter*
FQ used (n = 22)	0.86	0.36
FQ never used (n = 66)	0.50	0.17
Relative Risk (95% C.I.)	1.73 (1.29 – 2.32)	2.18 (1.01 – 4.72)

Table 3: Estimated adjusted odds ratios, with confidence intervals (C.I.s), of variables included as risk factors in the final logistic regression model for the occurrence of fluoroquinolone (FQ)-resistant *E. coli* on poultry farms

Risk Factor	co-efficient	p-value*	Lower Limit C.I.s 95%	Lower Limit C.I.s 90%	Odds ratio point estimate	Upper Limit C.I.s 90%	Upper Limit C.I.s 95%
Constant	- 0.204	0.6294					
Use of FQ in the past	2.049	0.0016	1.85	2.31	7.76	26.04	32.48
Site operated single-handedly	0.948	0.073	0.89	1.06	2.58	6.30	7.46
Site enclosed by a perimeter fence	- 1.302	0.014	0.09	0.11	0.27	0.67	0.79
Site has public footpath on the perimeter	1.407	0.019	1.17	1.43	4.09	11.67	14.20

n = 83; maximum re-scaled r^2 = 29.9%

*p-value is based on likelihood ratio test.

Table 4: Estimated adjusted odds ratios of variables, with confidence intervals (C.I.s), of variables included as risk factors in the final logistic regression model for the occurrence of fluoroquinolone (FQ)-resistant *Campylobacter* spp. on poultry farms

Risk Factor	co-efficient	p-value*	Lower limit C.I.s 95%	Lower limit C.I.s 90%	Odds ratio point estimate	Upper limit C.I.s 90%	Upper limit C.I.s 95%
constant	1.476	0.2387					
Use of FQ at any time in past	2.685	0.0052	1.64	2.32	14.65	92.59	130.59
No. of birds on site higher than median	- 2.182	0.0097	0.02	0.024	0.11	0.54	0.73
Site owned by an independent grower	- 3.156	0.0031	0.00	0.005	0.04	0.36	0.54
Masks provided for staff	- 1.412	0.081	0.05	0.062	0.24	0.96	1.24
All detailed areas are dusted	- 2.147	0.0089	0.02	0.026	0.12	0.52	0.69
Feed hoppers cleaned and disinfected	- 1.684	0.061	0.03	0.041	0.19	0.85	1.13
Wild birds have access to poultry houses	2.332	0.017	1.40	1.91	10.30	55.46	76.05

n = 84; maximum re-scaled r^2 = 56.3%

*p-value is based on likelihood ratio test.