Risk factors for the occurrence of *Escherichia coli* virulence genes *eae*, *stx1* and *stx2* in wild bird populations

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SUMMARY

Shiga toxin-producing *Escherichia coli* (STEC) can cause serious disease in human beings. Ruminants are considered to be the main reservoir of human STEC infections. However, STEC have also been isolated from other domestic animals, wild mammals and birds. We describe a cross-sectional study of wild birds in northern England to determine the prevalence of *E. coli*-containing genes that encode Shiga toxins (*stx1* and s*tx2*) and intimin (*eae*), important virulence determinants of STEC associated with human disease. Multivariable logistic regression analysis identified unique risk factors for the occurrence of each virulence gene in wild bird populations. The results of our study indicate that while wild birds are unlikely to be direct sources of STEC infections, they do represent a potential reservoir of virulence genes. This, coupled with their ability to act as long-distance vectors of STEC, means that wild birds have the potential to influence the spread and evolution of STEC.

Key words: Multivariable model, Shiga-toxin producing *E. coli*, wild birds.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) can cause a range of serious disease syndromes in humans, including diarrhoea, haemorrhagic colitis and life-threatening, haemolytic–uraemic syndrome (HUS) [1]. Transmission of STEC can be foodborne, waterborne or from person to person [2]. Ruminants, in particular cattle, are considered to be the main reservoir of human STEC infections, in particular those caused by serogroup O157 [3]. However, STEC have also been isolated from other domestic animals, wild mammals and birds [4–6] and Shiga toxin-production genes (*stx*) have also been found in a range of bacteria in the environment [7].

Two types of Shiga toxin, stx1 and stx2 (encoded by *stx1* and *stx2* genes), are associated with human disease. These toxins vary in their amino-acid sequence [8], antigenicity, and in their activation and receptor specificity [9]. *E. coli* acquire *stx* genes, and the subsequent ability to produce toxins, following infection with temperate bacteriophages [10].

The ability of *E. coli* to adhere to intestinal epithelium is crucial in the colonization of the intestine, and therefore the progression of disease in humans. The protein intimin, encoded by the *eae* gene, enables

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intimate attachment of *E. coli* to intestinal cells [11], causing characteristic attaching/effacing lesions [12]. This attachment also enables Shiga toxins to be injected into the epithelial cytoplasm through a type III secretion system [13].

Although wild birds have the potential to play a role both as reservoirs and long-distance vectors of STEC (and therefore *stx* and *eae* genes), studies of STEC in wild bird populations have been limited to either surveys for the O157 serogroup of *E. coli* [6, 14, 15], or to a limited range of wild bird species [16–18]. The aim of this study was therefore to investigate the prevalence, host range and associated risk factors for the occurrence of STEC and the virulence genes *stx1*, *stx2* and *eae* in *E. coli*, in a wider range of British wild bird populations. *Stx* genes are known to be transmitted horizontally between *E. coli* by highly mobile bacteriophages [10], therefore investigating the occurrence and distribution of these genes is of great importance to better understand the complex epidemiology and evolution of STEC.

METHODS

Study design

Serial cross-sectional surveys of wild bird populations throughout northern England were undertaken between July 2004 and October 2006. Samples were collected from a large range of wild bird species including representatives from the majority of wild bird families that are present in the UK for part of (migratory species) if not the entire year (resident species). Wild bird individuals were the basic sampling unit.

Sample sources

Samples were collected from both live and dead wild birds. In the UK, live wild birds can only be caught by persons holding a licence issued by the Department for Environment, Food and Rural Affairs (Defra), through a bird ringing scheme run by the British Trust for Ornithology (BTO). All live wild birds sampled in this study were trapped in collaboration with licensed BTO bird ringers [19]. A variety of methods were employed to catch wild birds including mist netting, cannon netting, ground traps, wildfowl round-ups, wildfowl shooting, sampling nestlings in the nest, and environmental sampling.

Dead garden birds were collected as part of a collaborative project between the Institute of Zoology and the University of Liverpool; the Garden Bird Health Initiative (GBHi), a study investigating causes of garden bird mortality [20].

Sample collection

Once captured, each live bird was placed in a paper bag [21], in which they usually defecated allowing a faecal sample to be collected. A new paper bag was used for each bird. Faecal samples were collected from the bags with sterile cotton-tipped swabs, placed in bacterial transport media (Medical Wire and Equipment Ltd, UK) and were transported to the laboratory in a cool box. If the bird did not defecate in the bag, or the bird was too large to place in a bag, a cloacal swab was taken. Occasionally, faecal samples were collected from the environment with sterile swabs as described above. Faecal samples were also collected from the lower intestine of dead birds during post-mortem examination.

Data collection

Data were recorded in the field about the bird and the site at which the bird had been caught or found. The date, location and habitat type where the bird had been trapped or found dead were recorded as well as the capture method, BTO ring number, species, age and sex.

The age and sex of a bird were determined by looking at a combination of features often specific to each species including plumage characteristics, presence or absence of a moult limit, and the size of the bird. When possible, birds were assigned to an age category using the BTO recommended age codes [19]. Depending on the species or time of year, not all of the measurements could be taken.

Isolation of *E. coli*

About 0·5 g faecal material from each bird, or the cloacal swab were placed in 2 ml of brain-heart infusion broth (LabM, UK) containing 5% glycerol (Sigma-Aldrich, UK) and homogenized by vortexing. Five hundred microlitres of the faecal suspension were added to 4 ml buffered peptone water that was incubated aerobically at 37 °C for 24 ± 4 h. A loop of the buffered peptone water was subcultured onto eosin Methylene Blue agar (EMBA; LabM), which was incubated aerobically for 20–24 h at 37 °C. Ten colonies from each agar plate with a metallic green

morphology typical of *E. coli* were frozen in 2-ml tubes filled with small beads in glycerol (Pro-Lab Diagnostics, UK) at $-80\ ^\circ$C until they were required.

Examination for virulence genes

Isolates were screened by polymerase chain reaction (PCR) for the presence of STEC virulence genes *eae*, *stx1* and *stx2*. DNA was prepared directly from colonies that had been frozen at $-80\ ^\circ$C. Crude DNA extracts were prepared for each isolate by boiling one bead in 0·5 ml sterile distilled water for 20 min in a 1·7-ml microcentrifuge tube. Cell lysates were kept at $4\ ^\circ$C for no longer than 14 days. 'Reddy mix' PCR master mix (ABgene) was used for the PCR with a final reaction volume of $25\ \mu$l. The final concentrations of the components in the PCR mix were 1·25 U *Taq* DNA polymerase (ABgene), 75 mM Tris–HCl, 20 mM $(NH_4)SO_4$, 1·5 mM $MgCl_2$, 0·01 % (v/v) Tween-20, 0·2 mM each of dATP, dCTP, dGTP and dTTP, 10 pmol/μl each primer and 1 μl DNA template. The presence of the intimin gene (*eae*) was investigated using primer sequences forward (sequence 5′ to 3′); GCTTAGTGCTGGTTTAGGATTG and reverse CCAGTGAACTACCGTCAAAG [22, 23]. *Stx* genes were detected using the primers *slt1*-F, CGCTGTTGTACCTGGAAAGG; *slt1*-R, CGCTCTGCAATAGGTACTCC [24], *slt2*-F, GCTTCTGCTGTGACAGTGAC; *slt2*-R, TCCATGACAACGGACAGCAG [24]. The PCR reaction was carried out with an initial denaturation step at $94\ ^\circ$C for 2 min followed by 25 cycles of amplification (denaturation at $94\ ^\circ$C for 1 min, annealing at $62\ ^\circ$C for 1 min 30 s and extension at $72\ ^\circ$C for 2 min). A strain of *E. coli* serotype O157, isolated from cattle faecal samples and known to be positive for *eae*, *stx1* and *stx2* genes, was used as a positive control for each of the PCR assays. PCR products were separated by gel electrophoresis using a 1·5% agarose gel in Tris–acetate buffer. Ethidium bromide was added to the agarose, and the gel was visualized under ultraviolet light. Product sizes were 625 bp, 250 bp and 190 bp, respectively, for *eae*, *stx1* and *stx2*.

Descriptive statistics

The prevalence of *E. coli* and each of the virulence genes, and their 95% confidence intervals (CI) were determined using the FREQUENCY command in the statistical software package EpiInfo 2002 (CDC, USA).

Univariate analysis

In all analyses, individual wild birds were the analytical units. Univariate and multivariable analyses were carried out using three different binary dependent variables: (1) the occurrence of *E. coli* that possessed the *stx1* gene; (2) the *stx2* gene and (3) the *eae* gene. Univariate associations between binary explanatory variables and the three dependent variables were investigated by performing univariable χ^2 tests or Fisher's exact tests (for expected values <5) in Stata v.8 (Stata Corporation, USA). Univariate relationships between the dependent variables and independent categorical variables were examined using χ^2 tests for differences between groups and χ^2 tests for trend in Stata v.8. Data from wild bird species from which fewer than 10 individuals were sampled were excluded from the analysis due to small sample size.

Multivariable analysis

Multivariable logistic regression models were built for each dependent variable using maximum-likelihood estimation. The location at which birds were caught or found was included in the models as a random effect to account for the possibility that birds caught at the same location were more likely to be similar to each other than birds caught at different locations. The models were built using the XTLOGIT command in Stata v.8. After univariable screening, variables showing an association with the outcome variable with a *P* value of $<0\cdot25$ were considered for inclusion in the multivariable model [25]. Variables with $>10\%$ missing values were not entered into the final models to avoid numerical instability. Simple correlation analysis was performed on independent binary variables. Categorical variables were assessed for correlation using the Cramer's phi-prime test statistic, which was carried out in SPSS v.15.0 (SPSS Inc., USA). Associations were considered statistically significant at a *P* value $\leqslant 0\cdot05$. Where two categorical variables were significantly associated and the Cramer's phi-prime test statistic value was $>0\cdot3$, the directional measure lambda was calculated to provide an indication of how strong the association between each categorical variable was. Statistically significant associations between two categorical variables with a lambda value of $>0\cdot3$ were considered to be strongly associated and therefore correlated. Of the correlated variables, those with the strongest association with the outcome variable or those variables that made most biological sense were considered for inclusion in

the final model to avoid covariance between predictor variables.

The final models were built manually. Variables were entered individually; a maximum-likelihood test ($P < 0.05$) and the model deviance were used to assess how well parameters improved the fit of the model. Parameters with a term-wise Wald test statistic of $P < 0.05$, and parameters that significantly improved the fit of the model (maximum-likelihood χ^2 test statistic $P < 0.05$) were retained in the final model. Once each final model had been built, all explanatory variables were re-entered individually to ensure that there were no more significant improvements.

The final models were evaluated for confounding by observing changes in parameter coefficients during the model-building process. Two-way interaction terms were tested between model parameters. For random-effects multivariable models, the number of quadrature points used in the random-effects estimator was checked for stability using the QUADCHK function in Stata v.8. Stata uses Gauss–Hermite quadrature approximation when estimating random-effects models. Twelve quadrature points are used by default when models are being built. To determine the soundness of quadrature approximation, the model was re-estimated using different numbers of quadrature points (8 and 16). The log-likelihood and co-efficient estimates for the original and re-estimated models were compared and a relative difference of $< 1\%$ was taken as evidence that the choice of quadrature points used did not significantly affect the outcome. To assess the percentage of variation that was attributable to the level of the location at which the bird was sampled, the intra-class correlation coefficient was calculated using a latent variable approach [26]. In models where the random effect was not statistically significant, a logistic regression model was built and the fit of the final model was assessed by examining the model sensitivity and specificity [area under the receiver operating characteristic (ROC) curve] and the Hosmer–Lemeshow test statistic.

RESULTS

Descriptive epidemiology

Faecal samples were collected from 2084 wild birds of 99 different species from 167 locations between July 2004 and October 2006. The overall prevalence of presumptive *E. coli* isolated from faecal samples was 50·8% (95% CI 48·6–52·9%), isolated from 94 bird
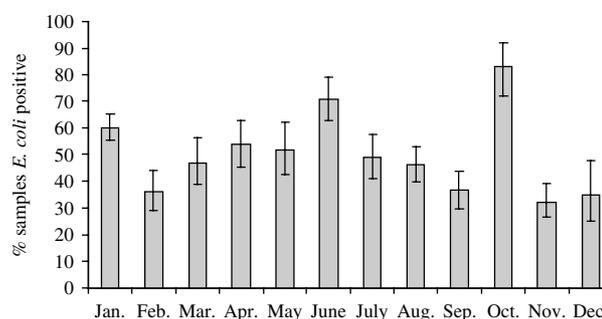

Fig. 1. Monthly prevalence estimates of *E. coli* isolated from wild bird faecal samples. Ninety-five percent confidence intervals are shown.

species. Of those samples from which *E. coli* was isolated, the prevalence of *stx1*, *stx2* and *eae* genes was 1·5% (95% CI 0·9–2·5), 7·9% (95% CI 6·4–9·8) and 4·9% (95% CI 3·7–6·4), respectively. The *stx1* genes were isolated from 12 wild bird species, *stx2* genes from 30 species and *eae* genes from 27 species of wild bird. There were significant differences in the monthly prevalence of *E. coli* ($P < 0.001$), both virulence genes *stx1* ($P = 0.02$) and *stx2* ($P < 0.001$) but not *eae* ($P = 0.17$). In the majority of samples, *eae*, *stx1* or *stx2* genes were detected individually; however, in 0·4% (95% CI 0·2–1·0) of samples both *stx1* and *eae* genes were detected, and in 0·2% (95% CI 0·05–0·7) of samples both *stx2* and *eae* genes were detected. It should be noted that multiple virulence genes were not necessarily derived from the same *E. coli* isolate as a pool of 10 *E. coli* isolates that were cultured from each faecal sample were screened by PCR. *E. coli* was most prevalent during January, June and October (Fig. 1). The *stx1* gene was detected most frequently in *E. coli* isolated in June whereas *stx2* showed peaks of occurrence in February, June, September and October (Fig. 2).

Multivariable analysis

Risk factors for the occurrence of E. coli *containing* stx1, stx2 *and* eae *genes*

The location at which birds were sampled was included in all multivariable logistic regression models as a random effect to account for the potential clustering effect. However, the proportion of the variation attributable to sampling location was negligible in all cases.

Table 1 shows the final multivariable logistic regression model of risk factors associated with the occurrence of *E. coli* in which *stx1* genes were detected.
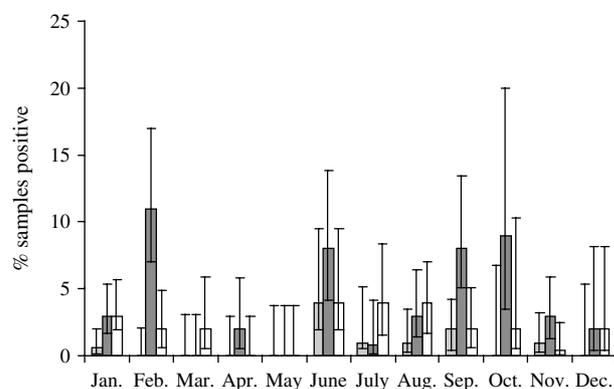
Fig. 2. Monthly prevalence estimates of *E. coli* isolated from wild bird faecal samples possessing virulence genes *stx1* (□), *stx2* (■) and *eae* (□). Ninety-five percent confidence intervals are shown.

Table 1. *Factors associated with the isolation of* E. coli *possessing the virulence gene* stx1 *from wild bird faecal samples as determined by multivariable logistic regression analysis* (n = 1196)

Variable	OR	95% CI	*P* value
Year sampled			
2004	Ref.	—	—
2005	4·58	1·00–21·65	0·05
2006	—	—	—
Season sampled			
Winter (Dec.–Feb.)	Ref.	—	—
Spring (Mar.–May)	—	—	—
Summer (June–Aug.)	6·65	1·38–32·10	0·02
Autumn (Sept.–Nov.)	4·06	0·69–23·81	0·12
Salmonella spp. isolated from faecal samples	12·93	1·32–125·98	0·03
E. coli possessing *eae* gene isolated from faecal samples	15·20	4·32–53·50	<0·001

OR, Odds ratio; CI, confidence interval.

The occurrence of *stx1* genes was associated with the year during which samples were collected: the odds of isolating *E. coli* possessing *stx1* genes from faecal samples were more than four times greater in 2005 than in 2004, and *stx1* genes were not detected in *E. coli* isolated during 2006. There were increased odds that *E. coli* positive for *stx1* genes were isolated from faecal samples collected during summer (June–August inclusive) compared to winter (December–February inclusive). Faecal samples from which *Salmonella* was isolated were more likely to yield *E. coli* containing *stx1* genes. In addition, there was a positive association between the occurrence of *stx1*

Table 2. *Factors associated with the isolation of* E. coli *possessing the virulence gene* stx2 *from wild bird faecal samples as determined by multivariable logistic regression analysis* (n = 1748)

Variable	OR	95% CI	*P* value
Season sampled			
Winter (Dec.–Feb.)	Ref.	—	—
Spring (Mar.–May)	0·07	0·01–0·32	0·001
Summer (June–Aug.)	0·52	0·25–1·08	0·08
Autumn (Sept.–Nov.)	2·35	1·25–4·42	0·008
Taxonomic order			
Anseriformes	Ref.	—	—
Ciconiiformes and Gruiformes	2·66	0·53–13·30	0·23
Columbiformes	0·83	0·17–3·91	0·81
Passeriformes	0·26	0·13–0·52	<0·001
Falconiformes	6·20	1·10–34·96	0·04
Charadriiformes	5·31	2·50–11·28	<0·001
Strigiformes	—	—	—
Salmonella spp. isolated from faecal samples	4·25	1·04–17·38	0·04

OR, Odds ratio; CI, confidence interval.

genes and *eae* genes in *E. coli* isolated from faecal samples. Goodness-of-fit statistics provided evidence for a good model fit (Hosmer–Lemeshow $\chi^2 = 1·62$, $P = 0·81$; area under the ROC curve = 0·78).

The final multivariable logistic regression model of risk factors associated with the occurrence of *E. coli* possessing *stx2* genes is shown in Table 2. The odds of isolating *E. coli* containing *stx2* genes from faecal samples were significantly lower during spring (March–May inclusive) and significantly higher during autumn (September–November inclusive) compared to winter. Compared to samples collected from Anseriformes (ducks, geese and swans), samples collected from Passeriformes (passerines) had reduced odds of being positive for *E. coli* containing *stx2* genes; however, *stx2* genes were more likely to be detected in *E. coli* isolated from Falconiformes (falcons) and Charadriiformes (wading birds). *E. coli* containing *stx2* genes were more likely to be isolated from faecal samples from which *Salmonella* was also isolated. The Hosmer–Lemeshow diagnostic test statistic was 7·55 ($P = 0·18$) and the area under the ROC curve was 0·81 indicating that the model was a good fit.

The final multivariable model of risk factors associated with the occurrence of *E. coli* in which *eae* genes were detected is shown in Table 3. Compared to samples collected during 2004, there were significantly

Table 3. *Factors associated with the isolation of* E. coli *possessing the virulence gene* eae *from wild bird faecal samples as determined by multivariable logistic regression analysis (n = 1748)*

Variable	OR	95% CI	*P* value
Year sampled			
2004	Ref.	—	—
2005	0·53	0·22–1·32	0·17
2006	0·19	0·04–0·82	0·03
Season sampled			
Winter (Dec.–Feb.)	Ref.	—	—
Spring (Mar.–May)	0·23	0·04–1·21	0·08
Summer (June–Aug.)	1·38	0·56–3·39	0·48
Autumn (Sept.–Nov.)	0·29	0·10–0·89	0·03
Taxonomic order			
Strigiformes	Ref.	—	—
Anseriformes	0·76	0·14–4·09	0·75
Ciconiiformes and Gruiformes	1·13	0·12–10·36	0·91
Columbiformes	0·34	0·03–4·00	0·39
Passeriformes	0·20	0·05–0·85	0·03
Falconiformes	—	—	—
Charadriiformes	0·19	0·03–1·36	0·10
E. coli possessing *stx1* gene isolated from faecal samples	9·07	2·24–36·73	0·002

OR, Odds ratio; CI, confidence interval.

lower odds of isolating *E. coli* with *eae* genes from samples collected during 2006. *E. coli* containing *eae* genes were more likely to be present in samples collected in autumn compared to samples collected during winter. Passeriformes had lower odds of carrying *E. coli* with *eae* genes when compared to Strigiformes (owls). Last, *E. coli* containing *eae* genes were more likely to be isolated from faecal samples from which *E. coli* containing *stx1* genes were also detected. The Hosmer–Lemeshow diagnostic test statistic for the multivariable model was 7·84 ($P = 0·35$) and the area under the ROC curve was 0·73 indicating that the model was a good fit.

DISCUSSION

The occurrence of STEC has been reported in wild birds previously, but studies have tended to concentrate on detecting particular serotypes of *E. coli* known to be associated with disease in humans, e.g. O157:H7, or detecting virulence genes in a limited number of wild bird species. Consequently, little is known about the wider host range or prevalence of STEC virulence determinants in *E. coli* carried by wild bird populations. *E. coli* virulence genes, known to be associated with human disease, namely *stx1*, *stx2* and *eae* were found in wild bird samples at a prevalence of 1·5% (95% CI 0·9–2·5), 7·9% (95% CI 6·4–9·8) and 4·9% (95% CI 3·7–6·4), respectively. This is consistent with previous studies where *stx* genes have been detected in wild birds at a prevalence ranging from 1·6% to 12·5% and *eae* genes have been reported in wild birds at a prevalence ranging from 4·5% to 40·0% [16–18, 27–29]. Domestic animals, in particular cattle, are considered to be the main reservoir of human STEC infections [3]. The results of our study are comparable with those of a study carried out over the same time period and in the same locality as the present study, which detected *stx1*-, *stx2*- and *eae*-positive *E. coli* in cattle faecal samples at prevalences of 3·0%, 1·8% and 5·4%, respectively (A. Lahuerta-Marin, personal communication).

The risk factors associated with the occurrence of *stx1* and *stx2* genes in *E. coli* isolated from wild birds differed, suggesting that these virulence genes, which are transmitted between bacteria on highly mobile bacteriophages, may have differing ecologies and may not occur at random. The proportion of *E. coli* isolates in which *stx1* genes were detected varied significantly during the 3 years of the study and the occurrence of *eae* genes declined significantly each year. However, it is difficult to determine if these observed differences reflect trends, and if so what might drive such trends. Similar year-to-year variability has been reported in the occurrence of STEC serogroup O157 in cattle and it was concluded that studies carried out over a longer time period would be required to determine if the observed variation was a genuine trend [30]. Year on year variation in environmental factors and the dynamics of wild bird populations, *E. coli* and bacteriophages may all be involved. The interplay between such factors can be complex.

The occurrence of each virulence gene was associated with a different season in the final multivariable models. The odds of *E. coli* containing *stx1* genes being isolated from wild birds were almost seven times greater during summer (June–August) than during winter (December–February), whereas there were reduced odds of isolating *E. coli* containing *stx2* genes during spring (March–May) and increased odds during autumn (September–November). The association of *stx*-positive *E. coli* with particular seasons is difficult to explain. It could be that clonal STEC strains may have been circulating among wild bird populations during those seasons. It could also be that

other unidentified risk factors, for which season is acting as a proxy measure, influence the shedding patterns of *E. coli* containing *stx* and *eae* genes in wild bird populations. Again, these factors (e.g. diet) might affect the dynamics of *E. coli* infection and shedding and/or the dynamics of *stx*-encoding bacteriophages.

There are very few studies of the occurrence of STEC in wild birds that report any seasonal trend. A study of the occurrence of variant *stx2* gene *stx2f* in feral pigeons in Italy reported no association between the occurrence of *E. coli* possessing the *stx2f* gene and season [17]. However, another study reported a higher frequency of *eae* genes in *E. coli* isolated from rock pigeons in Colorado, USA, during summer and autumn compared with winter [16]. This contrasts with the findings of the present study where the odds of detecting *eae* were significantly less during autumn than in winter. As already mentioned, there are likely to be complex interactions between hosts (different birds and different environments) and microorganisms (strains of *E. coli* and bacteriophages carrying *stx* genes), which give rise to the observed seasonal patterns that are likely to vary over time.

Multivariable logistic regression analysis revealed a significant association between the occurrence of *E. coli* containing *stx2* genes and the taxonomic order of the bird. No such association was found for the occurrence of *stx1*-positive *E. coli*. *Stx2*-positive *E. coli* were detected in faecal samples collected from 30 species of wild bird. However, they were most strongly associated with samples collected from Charadriiformes (wading birds) and Falconiformes (falcons). Most wading birds commonly feed in estuaries and it is possible that estuarine habitats are a source of *stx2*-positive *E. coli*, which birds may acquire through ingestion: 84% of birds sampled in an estuarine habitat in this study were Charadriiformes. It was not possible to enter the habitat in which birds were sampled into the final multivariable model as there was a correlation between the habitat in which birds were sampled and the taxonomic order. However, univariable analysis showed that *stx2* genes were most commonly detected in *E. coli* isolated from birds sampled in estuarine habitats (data not presented).

The increased frequency of *stx2*-positive *E. coli* from birds sampled in estuarine habitats could be a result of such bacteria being present in rivers that discharge into estuaries. Previous studies have reported the occurrence of *stx2* genes in both coliform populations [31] and as free bacteriophages [32] in aquatic environmental samples, including rivers. Earlier studies showed that waterways can be contaminated with *E. coli* containing virulence genes from slaughterhouse wastewater [33] and by direct faecal contamination by livestock [34].

Under certain conditions, such as a lack of nutrients and ultraviolet light irradiation, temperate lambdoid bacteriophages present within *E. coli* can be induced from the lysogenic cycle into the lytic cycle causing the release of infectious bacteriophages [7]. It is possible that the conditions required to induce *stx2*-bacteriophages into the lytic cycle are provided by estuarine habitats resulting in the release of free bacteriophages into the environment. The results of several studies indicate that *stx2*-bacteriophages have a diverse host range of *E. coli* strains; therefore, estuarine habitats may be a source of *stx2*-gene-containing *E. coli* and infectious bacteriophages, which birds acquire through feeding.

The association between *stx2*-positive *E. coli* with Falconiformes may also be explained by diet. Wild bird species that comprised the Falconiform group in this study included the common buzzard (*Bueto bueto*), common kestrel (*Falco tinnunculus*), peregrine (*Falco peregrinus*) and Eurasian sparrowhawk (*Accipiter nisus*). All of these birds are predatory and feed on small mammals, carrion, passerine birds, wading birds and/or pigeons. There are few studies that report STEC in wildlife species; however, *stx*-gene positive *E. coli* have been reported previously in rodents [27], rabbits [35], passerines [27], pigeons [17, 18, 29] and the present study found a strong association between *stx2*-positive *E. coli* and wading birds. It is likely that Falconiformes acquire *stx2*-gene-carrying *E. coli* via the oro-faecal route from their prey species.

Likewise the association between the occurrence of *E. coli* containing *eae* genes and Strigiformes (owls) may be diet related. The final logistic regression model showed that *eae* gene-positive *E. coli* were less likely to be isolated from Passeriformes (*eae* gene detected in 2% of samples) than from Strigiformes (*eae* gene detected in 19% of samples). The Strigiform group in this study was comprised mainly of barn owls (*Tyto alba*), which have a diet primarily of small rodents. A recent study that investigated the role of wildlife, including mammals and birds, in the transmission of STEC to livestock found a 5% prevalence of *eae*-positive *E. coli* in wild rodents (A. Lahuerta-Marin, personal communication). Barn

owls generally swallow their prey whole providing a mechanism for the acquisition of *eae*-positive *E. coli* harboured in the intestines of their rodent prey.

Multivariable logistic regression revealed that *stx1*- and *stx2*-positive *E. coli* were more likely to occur in faecal samples that also contained *Salmonella enterica*. Salmonellae are yet to be associated with phage-related disease and it is therefore difficult to explain this association. It is could be that other unidentified risk factors, for which the occurrence of *S. enterica* is acting as a proxy, influence the occurrence of *stx*-positive *E. coli* in wild bird populations. For example, intestinal conditions that are favourable for the presence of *E. coli* carrying *stx* genes may also be favourable for the presence of *S. enterica*. Alternatively, wild birds from which both *S. enterica* and *stx*-positive *E. coli* were isolated may have acquired both bacteria from one source, e.g. livestock. Asymptomatic infection with both *S. enterica* and STEC is known to occur in some species of livestock, in particular ruminants [3, 36].

The occurrence of the *stx1* gene in *E. coli* isolated during the present study was highly associated with the occurrence of the *eae* gene. This association is difficult to explain as *stx1* genes are encoded on bacteriophages whereas the *eae* gene is located on the LEE pathogenicity island on the bacterial chromosome. However, in a study investigating the prevalence of the *eae* gene in STEC strains from dairy cattle, *eae*-positive isolates were also more likely to be positive for *stx1* [37].

In summary, we have shown that *E. coli* containing genes that encode Shiga toxin (*stx1* and *stx2*) and intimin production (*eae*), which are important virulence determinants in *E. coli* associated with human disease, can be detected in a range of wild bird species in Great Britain. The *stx2* gene was the most commonly detected virulence gene, and this has been shown by others [12, 38] to be more strongly associated with severe human disease than *stx1* genes. Wild bird populations represent a potential reservoir of virulence genes that are known to be transmitted horizontally between *E. coli* strains on highly mobile temperate lambdoid bacteriophages. This, coupled with the ability to act as long-distance vectors of STEC, means that wild birds have the potential to influence the spread and evolution of STEC. Logistic regression analysis identified several unique risk factors for the occurrence of each virulence gene, which may suggest these virulence genes do not occur at random and the mobile genetic elements that encode virulence genes have individual and differing ecologies.

DECLARATION OF INTEREST

None.

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