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Citation for published version:
O'Reilly, KM, Low, JC, Denwood, MJ, Gally, DL, Evans, J, Gunn, GJ, Mellor, DJ, Reid, SW & Matthews, L

Digital Object Identifier (DOI):
10.1128/AEM.01343-10

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Applied and Environmental Microbiology

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Associations between the Presence of Virulence Determinants and the Epidemiology and Ecology of Zoonotic Escherichia coli

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Received 5 June 2010/Accepted 16 September 2010

The severity of human infection with pathogenic Escherichia coli depends on two major virulence determinants (eae and stx) that, respectively, produce intimin and Shiga toxin. In cattle, both may enhance colonization, but whether this increases fitness by enhancing cattle-to-cattle transmission in the field is unknown. In E. coli O157, the almost uniform presence of the virulence determinants in cattle isolates prevents comparative analysis. The availability to this study of extensive non-O157 E. coli data, with much greater diversity in carriage of virulence determinants, provides the opportunity to gain insight into their potential impact on transmission. Dynamic models were used to simulate expected prevalence distributions for serogroups O26 and O103. Transmission parameters were estimated by fitting model outputs to prevalence data from Scottish cattle using a Bayesian Markov chain Monte Carlo (MCMC) approach. Despite similar prevalence distributions for O26 and O103, their transmission dynamics were distinct. Serogroup O26 strains appear well adapted to the cattle host. The dynamics are characterized by a basic reproduction ratio (R₀) of >1 (allowing sustained cattle-to-cattle transmission), a relatively low transmission rate from environmental reservoirs, and substantial association with eae on transmission. The presence of stx₁ was associated with reduced transmission. In contrast, serogroup O103 appears better adapted to the noncattle environment, characterized by an R₀ value of <1 for plausible test sensitivities, a significantly higher transmission rate from noncattle sources than serogroup O26, and an absence of fitness benefits associated with the carriage of eae. Thus, the association of eae with enhanced transmission depends on the E. coli serogroup. Our results suggest that the capacity of E. coli strains to derive fitness benefits from virulence determinants influences the prevalence in the cattle population and the ecology and epidemiology of the host organism.

Human-pathogenic Escherichia coli may be categorized into different pathogen types that include enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC) (26). The primary distinction is the ability of EHEC strains to produce Shiga toxin (Stx) due to the integration into the genome of a bacteriophage carrying the encoding gene (stx). Central to the pathogenesis of EPEC is its ability to colonize the intestinal mucosa by the formation of attaching and effacing (A/E) lesions (26). A key component of this complex adhesion system, which may be possessed by both EPEC and EHEC strains, is the production of intimin, encoded by the eae gene. The genes required for the formation of A/E lesions are carried on a 36-kb pathogenicity island termed the locus of enteroocyte effacement (LEE) (24).

Cattle may carry high densities of these E. coli pathogen types, including those that cause serious human infections, such as E. coli O157. E. coli serogroups O26, O103, O145, and O111 have also emerged as increasingly important causes of human infection, and in some countries non-O157 serogroups now dominate E. coli serogroup O157 as a source of human infection (4, 16). These strains may often be asymptotically carried by cattle (6, 10, 30, 37), yet intriguingly there is widespread carriage of virulence genes in cattle isolates of both O157 and non-O157 E. coli serogroups (13, 27).

Multilocus sequence typing (MLST) has shown that these E. coli pathogen types occur in many unrelated lineages and that EHEC strains are almost as closely related to EPEC strains as to other EHEC strains (41). The conclusion of these studies is that horizontally transmitted virulence genes may have been acquired independently on multiple occasions by EHEC and EPEC strains. As the horizontal acquisition of virulence is presumably under a selection pressure exerted by the host, driving bacterial mutation and recombination (41), the finding raises questions as to the function of these virulence determinants in the ecology and epidemiology of the strains in cattle.

Experimental work has shown that the virulence determinant eae can enhance colonization by E. coli O157 in ruminants (7, 8, 9, 31) and may increase shedding of the pathogen (7). It remains to be established whether this leads to enhanced transmission of the pathogen in the field. The role of Shiga toxin is less well understood. Historically, the lack of apparent clinical infection and pathology in cattle was attributed to a lack of vascular receptors for a critical toxin, Stx₁ (33). However, recent work has shown the importance of Shiga toxin for adherence in culture systems and mice via nucleolin upregulation...
and binding to intimin (35). In cattle, Stx2 has been demonstrated to enhance colonization of intestinal tissues (2, 3, 20), demonstrating for the first time a direct role of a cytotoxin in colonization of cattle. To date, the role of these virulence determinants has been assessed in vitro or within individual hosts, but as far as we are aware, their potential impact on transmission in the field has not been investigated.

A recent survey of the Scottish cattle population determined the prevalence and distribution of the E. coli serogroups O157, O26, and O103 and their virulence genes eae and stx (28). Serogroup O157 isolates were extremely uniform, almost all being stx2 and eae positive (12), making it difficult to ascertain selective benefits to their presence. In contrast, the serogroup O26 and O103 isolates exhibited much greater diversity (28), which provides us with the opportunity to conduct comparative analyses of the transmission dynamics of strains with and without these key virulence determinants. Here, we use extensive cross-sectional data obtained from a prevalence survey of non-O157 E. coli (28) to quantify the transmission dynamics of the non-O157 strains.

The transmission of infection from individual to individual can be quantified in terms of the basic reproduction number, $R_0$, defined as the average number of secondary cases generated by a single infected individual in a naïve population (1). In large populations, if $R_0 > 1$, the infection on average will spread; if $R_0 < 1$, it is expected to decline. With small groups of individuals, it is recognized that stochastic or chance events will allow chains of infection to become extinct, requiring reintroduction for persistence of infection (17, 34).

Here, we quantify the transmission dynamics of the non-O157 strains in terms of the within-group $R_0$ and an immigration rate, $\lambda$, which captures transmission arising from movements of infected animals into the cattle management group or transmission from an environmental reservoir. We use the term environmental reservoir to refer to noncattle sources of infection that could also include other livestock or wildlife species. This allows us to quantify and contrast the mechanisms of persistence for serogroups O26 and O103 and to examine associations between the carriage of virulence genes and the strains’ ecology and epidemiology. An improved understanding of the potential role of virulence determinants of human-pathogenic E. coli in the cattle reservoir may inform the prediction of the emergence of future virulent strains.

**MATERIALS AND METHODS**

**Prevalence data.** A national survey was carried out to determine the prevalence of E. coli serogroups O26, O103, O111, and O145 in feces of Scottish cattle between March 2002 and February 2004 (28). Only serogroups O26 and O103 were included in our analysis, as the prevalence of the other serogroups was either low (serogroup O145) or zero (serogroup O111). In total, 6,086 fresh fecal pats from 338 farms were tested. The 338 farms contained 414 groups of cattle that were managed separately. In each individual management group, sufficient fecal pats from the sampled farms were tested to ensure an 80% probability of identifying at least one positive pat when there was at least one shedding animal within the group. Of the 414 groups, O26 was isolated in 72 groups and O103 in 78. The observed cattle level prevalence was 4.1% for O26 and 2.8% for O103 (Fig. 1 shows the observed prevalence distributions). All isolates were additionally screened for the virulence genes encoding intimin (eae), Shiga toxin 1 (stx1), and Shiga toxin 2 (stx2) (28). In addition, a related virulence determinant that produces enterohemolysin (ehxA) and which is also associated with severe human disease was screened for, but as its presence was very closely correlated with the presence of eae, we did not consider it further (28). Table 1 shows the breakdown of the isolates by virulence gene carriage.

**Strain definition.** The presence of the virulence determinant stx was not significantly associated with the presence of eae (28). Within the stx-positive O26 groups, strains could be further classified as being positive for stx1 and stx2 or positive for stx1 only. We found the percentage of stx1- and stx2-positive isolates that were positive for eae (80.2%) not to be significantly different from the percentage of stx1-only strains that were positive for eae (77.4%).

Our analyses investigated the transmission dynamics of the following strain combinations: (i) eae-positive or -negative strains, which are defined as those being positive/negative for eae irrespective of the stx presence; (ii) stx-positive or -negative strains, stx1- and stx2-positive or -negative strains, and stx1-only-positive or -negative strains, which are defined as those being positive/negative for the stx combination irrespective of the eae presence; and (iii) eae-positive and stx-positive or -negative strains. Note that the isolate numbers were too small to

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**FIG. 1.** Observed prevalence distributions for E. coli serogroups O26 (black) and O103 (gray) derived from a sample of 6,086 fecal pats from 414 cattle groups. (a) Full dataset; (b) dataset excluding observed zero prevalences, to expose the tails of the distributions.
TABLE 1. Numbers of O26 and O103 strains and frequency by virulence determinant

<table>
<thead>
<tr>
<th>Strain</th>
<th>Virulence determinant</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx₁</td>
<td>stx₂</td>
</tr>
<tr>
<td>O26</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>O103</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total (%)</td>
<td>122 (49.0)</td>
<td>31 (12.4)</td>
</tr>
</tbody>
</table>

TABLE 2. Transitions in stochastic susceptible-infected-susceptible model

<table>
<thead>
<tr>
<th>Transition</th>
<th>Symbolic notation</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible to infected</td>
<td>(S, I) to (S - 1, I + 1)</td>
<td>λS + βS/N</td>
</tr>
<tr>
<td>Infected to susceptible</td>
<td>(S, I) to (S + 1, I - 1)</td>
<td>αI</td>
</tr>
</tbody>
</table>

where \( R_0 = \frac{β}{α} \) is the basic reproduction ratio and \( λ_s = \frac{β}{α} \) is the rate of immigration measured relative to the timescale for recovery.

(1) Mathematical modeling and parameter estimation. (i) Within-group dynamics. E. coli strain dynamics were used to simulate the endemic prevalence and distribution of infection of two groups across the population of livestock holdings. Following the modeling approach employed previously to describe E. coli O157 (21, 22, 23), within-farm transmission dynamics were described by a stochastic, individual-based susceptible-infected-susceptible model. Infections were assumed to arise in the susceptible population via two possible routes (Table 2): first, transmission from other infected individuals with rate \( β/N \), where \( β \) is the transmission rate, \( S \) the number of susceptible individuals, and \( N \) the group size, giving \( R_0 = \frac{β}{α} \), and second, immigration of infection (at a rate of \( λS \)) from some external source, which could represent either the presence of an environmental reservoir or the movement onto the farm of an already infected individual. Here, we use the term environmental reservoir to refer to noncattle sources of infection that could also include other livestock or wildlife species. Infected individuals were assumed to recover (at a rate of \( α \)) to the susceptible state.

The stochastic within-herd transmission dynamics can be described as follows in terms of the probabilities, \( p_r \), of there being infected (i) animals in a group of \( N \):

\[
\frac{dp_r}{dt} = σ(i + 1)p_r + \left( λ + \frac{β(i - 1)}{N}(N - i) \right) p_{i-1} - \left( σ(i + 1) + \frac{λi}{N}(N - i) \right) p_r \quad \text{for } i = 1, N
\]

Solving these equations gives the following expression for the equilibrium probabilities, \( p^{eq} \).

\[
p^{eq}_1 = \frac{\left( λ + \frac{β}{N}(N - i)p^{eq}_0 \right)}{α(i + 1)} \quad \text{for } i = 1, N
\]

\[
p^{eq}_0 = 1 - \sum_{i=1}^{N} p^{eq}_i \]

(2) associations between the carriage of virulence determinants and cattle-to-cattle transmission. Transmission parameters were also estimated for E. coli O26 and O103 strains defined by the presence/absence of the virulence determinants eae and stx. The presence of eae was associated with substantially enhanced transmission of E. coli O26 (Fig. 3a), with strains positive for eae having \( R_0 \) estimates greater than 1 and strains negative for eae having \( R_0 \) estimates that were predominantly less than 1. In contrast, there was no association between the presence of eae and the transmission dynamics of E. coli O103 (Fig. 3b).

Overall, the presence of stx genes (either stx₁ only or both) had no association with the transmission of E. coli O26 (Fig. 3c, black lines), and there was no evidence of any interaction between the stx₁- and eae-possessing strains (Fig. 3c). However, when the stx-possessing strains were broken down into strains with either stx₁ only or stx₂, and stx₁ together, a difference in \( R_0 \) estimates was observed (Fig. 3d). To ascertain whether this difference in \( R_0 \) values between the stx₁-only or stx₂-only and stx₁-and-stx₂ strains was significant, repeated sampling from the two posto-
rior distributions was used to generate 10,000 pairs of $R_0$ estimates for the two strains. Assuming a uniform prior on the test sensitivities and averaging across the range of test sensitivities, the overall probability of obtaining an $R_0$ estimate for the $stx_1$-and-$stx_2$ strains that was higher than an estimate for the $stx_1$-only strain was less than 2.3%. Thus, we conclude that $stx_1$-only strains have a slightly higher $R_0$ than strains possessing $stx_1$ and $stx_2$ ($P < 0.023$). Associations between the carriage of $stx$ and transmission of O103 strains could not be examined due to its very low occurrence.

**DISCUSSION**

This study quantified the transmission dynamics in the Scottish cattle population of two major non-O157 *E. coli* serogroups and sought to identify associations between carriage of virulence determinants and their ecology and epidemiology. Strains were characterized by the presence of the virulence genes *eae* and *stx*, which are associated with severe disease in humans but whose role in the cattle reservoir is incompletely understood. Fitting dynamic models to the prevalence data for *E. coli* serogroups O26 and O103 revealed distinct transmission dynamics, reflecting different ecologies and epidemiologies of the serogroups. Specifically, analysis of the transmission dynamics revealed that, despite similar prevalence distributions, the two serogroups are maintained by different balances between $R_0$, the measure of cattle-to-cattle transmission, and the immigration rate, $\lambda$, which captures either transmission arising from movements of infected animals into the cattle management group or transmission from an environmental reservoir. Here, the term environmental reservoir refers to noncattle sources of infection that could also include other livestock or wildlife species.

*E. coli* serogroup O26 appears well adapted to the cattle host, with transmission dynamics characterized by an $R_0$ value of $>1$, allowing sustained cattle-to-cattle transmission, and a low transmission rate from an environmental reservoir relative
to *E. coli* O103. In contrast, serogroup O103 appears to be better adapted to the noncattle environment, with an *R₀* value of <1 for plausible test sensitivities and a significantly higher environmental transmission rate than that of *E. coli* O26. Although our estimates for the immigration rate encompass both transmission from environmental reservoirs and introduction via infected animals moving into the management group, the lower prevalence of serogroup O103 relative to serogroup O26 strains in the national herd suggests that the differences in immigration rates arise from differences in transmission rates from environmental sources.

An alternative interpretation of these results leads similarly to the conclusion that O103 has improved survival in the noncattle environment. The alternative possibility is that apparent differences in immigration rates arise because our estimation procedure calculates the immigration rate relative to the timescale for recovery from infection, and these may differ between O103 and O26.

To examine this further, it is useful to consider the results of a previous comparative analysis of the transmission dynamics of serogroups O26 and O103 in calves (19). Liu et al. demonstrate no difference in the within-host periods of infection for O26 and O103. However, because transmission can occur via exposure to infected pats, the recovery period as defined in our model encompasses survival in the environment. Therefore, any differences in our recovery period would reflect differences in the short-term survival of infectivity. Thus, the observed differences in the immigration rate might therefore reflect a longer timescale for the decay of infectivity in the environment for O103. These two interpretations of the differences in immigration between serogroups lead to the conclusion that O103 has either improved survival or a greater presence in the noncattle environment.

The serogroups also differed in the proportion of isolates positive for *eae* (84% for *E. coli* O26 and 38% for *E. coli* O103).
and the extent to which eae was associated with enhanced transmission. The presence of eae was associated with a significant increase in the transmission of E. coli O26, corresponding to an increase in the basic reproduction ratio, R\text{e}_0, from less than to more than 1. For E. coli O103, however, no association was observed. Our observation that the serogroup with the higher prevalence of eae-positive isolates corresponds to the serogroup with enhanced transmission of eae-positive strains suggests that the eae presence may translate into real selective advantages in the field.

That the association between the eae presence and transmission depends on the serogroup indicates a role for additional factors, such as virulence determinants not studied here, or the presence of different eae variants. Studies to characterize the eae allele in different serogroups have identified the eae allele for E. coli O26 as ε1, while the eae allele for E. coli O103 is either ε or ε (5, 18, 32, 40). The different intimin types may be responsible for different host tissue tropisms (5), which may in turn contribute to enhanced transmission of some serogroups possessing specific alleles. Alternatively, as we did not measure the ability of strains from the two serogroups to form ΔE lesions, there may be differences in the regulation of eae in the different genetic backgrounds.

Associations between the carriage of Shiga toxins and transmission could not be clearly elucidated by this study. Overall, the possession of stx genes (either stx\text{1} or both) had no association with the transmission of E. coli O26. However, breaking down the strains into stx\text{1}-only and stx\text{1}-and-stx\text{2} strains showed that the stx\text{1}-only strains have a significant increase in the reproduction ratio, R\text{e}_0, compared to the stx\text{1}-and-stx\text{2} strains. As recent research has identified a potential role of stx\text{2} in colonization of the intestine (2, 3, 20), this result runs contrary to expectations and may indicate interactions between the strains arising, for example, from the presence of other virulence factors not examined by this study. We did not find any evidence of an interaction between the stx- and eae-possessing strains that might have been anticipated, given recent work that has shown the importance of Shiga toxin for adherence via binding to intimin (35). Associations between stx carriage and transmission were not assessed for E. coli O103 due to its very low occurrence (2 of 168 isolates), but this in itself may well be a consequence of an absence of any selective advantage in this serogroup.

Our results suggest the existence of strains with distinct modes of persistence. The serogroup O26 strains appear better adapted to the cattle host and able to derive fitness benefits from the virulence determinants responsible for severe human disease. In contrast, the serogroup O103 strains appear better adapted to the noncattle environment and lack the capacity for sustained cattle-to-cattle transmission. These strains may lack appropriate eae alleles or be missing other genetic determinants that allow the exploitation of eae. We recognize that this study has not considered the potential influence and role of other virulence factors possibly linked to specific serotypes. Our results suggest that the capacity to derive fitness benefits from virulence determinants influences their prevalence in the cattle population and the ecology and epidemiology of the host organism. By identifying the potential fitness benefits of these virulence determinants in the cattle host, our results may ultimately help inform the design of controls and predict emergence patterns for pathogenic human strains.

ACKNOWLEDGMENTS

We are grateful to the Scottish government, which funded a Centre of Excellence in Epidemiology, Population Health and Infectious Disease Control, and to the Foods Standards Agency (Scotland), which funded the data collection and analysis. L.M. is grateful to the Wellcome Trust for a Mathematical Biology Research Training Fellowship that funded the development of the quantitative approaches. The Scottish Agricultural College receives financial support from the Scottish Government’s Rural and Environmental Research Affairs Department (RERAD).

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