Predicting the public health benefit of vaccinating cattle against *Escherichia coli* O157

Louise Matthews[a,1], Richard Reeve[a,b], David L. Gally[c], J. Chris Low[d], Mark E. J. Woolhouse[e], Sean P. McAteer[c], Mary E. Locking[f], Margo E. Chase-Topping[e], Daniel T. Haydon[a], Lesley J. Allison[g], Mary F. Hanson[g], George J. Gunn[h], and Stuart W. J. Reid[i]

[a]Boyd Orr Centre for Population and Ecosystem Health, Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G61 1QH, United Kingdom; [b]The Pirbright Institute, Woking, Surrey GU24 0NF, United Kingdom; [c]Immunity and Infection Division, The Roslin Institute and [d]Royal (Dick) School of Veterinary Science, University of Edinburgh, Midlothian EH25 9RG, United Kingdom; [e]Centre for Immunity, Infection and Evolution, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom; [f]Health Protection Scotland, Glasgow G2 6QE, United Kingdom; [g]Scottish *E. coli* O157/VTEC Reference Laboratory, Department of Laboratory Medicine, Royal Infirmary of Edinburgh, Edinburgh EH16 4SA, United Kingdom; [h]SRUC, Edinburgh EH9 3JG, United Kingdom; and [i]Royal Veterinary College, Hertfordshire AL9 7TA, United Kingdom

Edited by Burton H. Singer, University of Florida, Gainesville, FL, and approved August 8, 2013 (received for review April 2, 2013)

Identifying the major sources of risk in disease transmission is key to designing effective controls. However, understanding of transmission dynamics across species boundaries is typically poor, making the design and evaluation of controls particularly challenging for zoonotic pathogens. One such global pathogen is *Escherichia coli* O157, which causes a serious and sometimes fatal gastrointestinal illness. Cattle are the main reservoir for *E. coli* O157, and vaccines for cattle now exist. However, adoption of vaccines is being delayed by conflicting responsibilities of veterinary and public health agencies, economic drivers, and because clinical trials cannot easily test interventions across species boundaries, lack of information on the public health benefits. Here, we examine transmission risk across the cattle–human species boundary and show three key results. First, supershedding of the pathogen by cattle is associated with the genetic marker *stx2*. Second, by quantifying the link between shedding density in cattle and human risk, we show that only the relatively rare supershedding events contribute significantly to human risk. Third, we show that this finding has profound consequences for the public health benefits of the cattle vaccine. A naïve evaluation based on efficacy in cattle would suggest a 50% reduction in risk; however, because the vaccine targets the major source of human risk, we predict a reduction in human cases of nearly 85%. By accounting for nonlinearities in transmission across the human–animal interface, we show that adoption of these vaccines by the livestock industry could prevent substantial numbers of human *E. coli* O157 cases.

zoonoses | cross-species transmission | 80–20 rule | one health

In the 30 years since *Escherichia coli* O157 was identified as a source of serious gastrointestinal illness, it has emerged globally. Infection can lead to death or life-long kidney damage, and it is a major cause of acute renal failure in children (1). In addition to devastating personal losses, the economic costs can be substantial—in the United States, the costs of healthcare, social care, and lost productivity come to around $600 million per year (2), whereas costs to the food industry from product recalls and reduced trade can run to tens of millions of dollars. Newly developed cattle vaccines could help tackle this problem at its source.

Cattle are the main reservoir for *E. coli* O157 and harbor the pathogen in their gastrointestinal tract without developing clinical disease. Although direct person-to-person spread can occur, people are usually infected by either consuming contaminated food and water or contact with livestock feces in the environment. Reducing occurrence in cattle is one route to control, but a lack of effective interventions before slaughter means that control currently relies heavily on good hygiene practices by food producers and individuals in the domestic kitchen and the simple personal hygiene measure of hand washing. Several preslaughter interventions have been tried, including altered diets, adding probiotics to feed, spraying cattle with bacteriophage, and vaccination (3). Of these interventions, vaccines have proven to be the most effective; in vaccine trials, both experimentally and naturally infected cattle show significant reductions in the frequency, duration, and intensity of *E. coli* O157 excretion in their feces (4–8).

Two vaccines are now available: one vaccine in Canada, where it is fully licensed but with little uptake by farmers, and two vaccines in the United States, where restricted licenses permit only limited use (9). The delay in fully licensing these vaccines highlights two challenges to control planning that are posed by zoonotic infections. First, there are conflicting responsibilities of the medical and veterinary agencies. The bodies charged with licensing vaccines in animals must typically certify that controls are not just safe but improve animal health; this remit poses a problem for the control of zoonotic pathogens that are benign in their reservoir hosts and demands a coordinated approach from medical and veterinary agencies (9–11). Second, controls applied in animals are not easily tested against the key outcome—the reduction in human illnesses—and this lack of data on impact hampers effective decision-making.

In Canada, the issue is gaining political momentum and media attention—a recent outbreak prompted their media to cite the failure to vaccinate as "irresponsible if not worse" (12). In the United Kingdom, the 2009 Godstone Farm (13) outbreak that caused 93 cases, most of whom were children, prompted interest in vaccination on open or petting farms. A special treatment certificate is now available, allowing open farms to apply for permission to vaccinate. However, whether vaccination would ever be widely used in the United Kingdom, North America, or the wider world will depend on the number of illnesses and deaths prevented; the cost of vaccination; and whether costs could be shared between the public, government, and food industry to help make vaccination a viable option for the farming community.

Disease control planning (when costs and benefits are distributed across several stakeholder groups) is a challenging problem but one that occurs frequently for zoonotic disease (14). A one health approach that integrates the understanding and management of human and animal disease has gained momentum in the last decade and been used to show that certain

Author contributions: L.M., R.R., D.L.G., J.C.L., M.E.J.W., M.E.L., G.J.G., and S.W.J.R. designed research; L.M., R.R., D.L.G., S.P.M., M.E.L., M.E.C.-T., and L.J.A. performed research; L.M., R.R., and M.E.C.-T. analyzed data; and L.M., R.R., D.L.G., J.C.L., M.E.J.W., M.E.L., M.E.C.-T., D.T.H., L.J.A., M.F.H., G.J.G., and S.W.J.R. wrote the paper.

Conflict of interest statement: D.L.G. supervises a studentship part-funded by Bioniche Animal Health examining immune response to EHEC (Enterohemorrhagic *E. coli*) O157 colonization. This project and funding source has not influenced the work presented in this manuscript. The other authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

[1]To whom correspondence should be addressed. E-mail: louise.matthews@glasgow.ac.uk.

interventions in livestock are not only effective but also cost-saving when costs and benefits are combined across multiple sectors (14–17). Narrod et al. (10) propose a five-step framework for estimating the costs and societal benefits of controlling zoonoses. Underpinning this framework are integrated models of disease transmission within the animal reservoir, disease transmission from reservoir to humans, and the impact of risk management strategies on the disease dynamics. Here, we focus on the understanding of disease ecology and cross-species transmission dynamics of *E. coli* O157 to tackle the question of the number of human cases prevented by vaccination.

In the absence of direct data, mathematical models of transmission across the human–animal interface provide tools to predict the success of interventions. However, few such models for zoonotic infections exist (18), a consequence of the difficulty in mapping the distribution of disease in the animal reservoir to incidence in the human population. The models that have been developed previously frequently assume linear relationships between prevalence in the animal host and human cases (15, 16). Zinsstag et al. (17), however, provide an example of transmission route heterogeneity captured in an animal to human rabies transmission model. In general, heterogeneities need to be captured when variation in host infectiousness, pathogen strain, and transmission route mean that crude prevalence in the animal reservoir may not be a good predictor of risk to humans. For *E. coli* O157, this issue is key. Cattle vaccines reduce the frequency of shedding by around 50% (6, 7), but how this translates into the reduction in human cases requires investigation of the cross-species transmission step to identify the factors promoting transmission across the species barrier.

Genetic and genomic studies have sought to identify the features of *E. coli* O157 strains in the cattle reservoir that appear most frequently in human cases (19, 20). These studies show that certain genetic markers, including variants of the Shiga toxin 2-producing genes (*stx2* and *stx2c*), are differentially associated with occurrence in humans (21). However, what is not delivered by these studies is an understanding of the epidemiological traits associated with these markers.

Determining how strains differ epidemiologically has important implications for the design and efficacy of control measures applied in cattle. If strains differ only in clinical severity after they have crossed the species barrier into humans, controls that reduce frequency of occurrence in cattle will produce proportionate reductions in numbers of human illnesses; if, however, strains differ in human risk because high shedding intensity boosts their capacity to cross the species barrier, then controls that reduce occurrence in cattle by reducing high-concentration shedding will be disproportionately effective at reducing human cases.

Consequently, a key issue is whether the Shiga toxin 2-producing gene variants are responsible for differences in epidemiology in the cattle reservoir or the severity of infection within the human host. Currently, their roles remain unclear. Although some studies have found associations between *stx2c* and more severe infections in people (22, 23), when examined in vitro, *stx2c* is found to produce a smaller quantity of less potent toxin than *stx2* (24). It has been argued, however, that, because humans play a small role in the epidemiology of the bacterium, the role of Shiga toxin may be to confer a selective advantage for the organism in cattle. Shiga toxin has been shown in mouse models to aid bacterial adherence and also dampen innate response signaling (25, 26). Another hypothesis is that Shiga toxin kills off predatory protozoa in the cattle gut (27). Consequently, the more frequent occurrence in humans of strains with particular *stx2* gene variants may be driven by differing epidemiology in the cattle host, affecting pathogen shedding and risk of transmission across the human–animal interface, rather than differing severity in humans after the species barrier has been crossed, affecting the likelihood of infections being reported.

In parallel with the genetic and genomic studies, epidemiological studies in the cattle reservoir have shown marked differences between strains in shedding intensity (the level of excretion of the pathogen in feces) (28, 29). Shedding at high intensities is important, because despite being relatively rare, it seems to be the major source of deposition into the environment; in our cattle data, shedding below 10^3 cfu/g feces was found in 86% of samples but accounted for less than 1% of the total bacteria shed (Fig. 1*B*). Such high-intensity shedding or supershedding is not confined to certain animals (30, 31) but viewed as an occasional phenomenon with the potential to occur in any individual animal, and it has been to shown to have an important role in amplifying cattle-to-cattle transmission (32–34). However, despite the attention that supershedding has received as a source of transmission between cattle, there are two important gaps. First, although phenotypic markers (phage type) exist, no genetic marker for supershedding has been found. Second, there has been no assessment of whether and to what extent supershedding might increase transmission risk to humans and how this phenomenon might be exploited for control.

This paper addresses both these gaps, with the goals of exposing the cross-species transmission dynamics and determining the public health benefits of cattle vaccination. We use extensive veterinary, human surveillance, and molecular data to quantify the relationship between pathogen shedding in the cattle reservoir and human risk, and we show that only the relatively rare supershedding events contribute significantly to human risk. Using this information, we show that, because the currently available vaccines target the high shedding densities—the major drivers of cross-species transmission—it produces a much greater reduction in human cases than would be predicted from its efficacy in the cattle reservoir.

Results

E. coli O157 strains from primary human cases and a large-scale cattle survey were used to ask whether supershedding strains were disproportionately frequent among human clinical isolates and determine the relationship between shedding density in the cattle reservoir and human risk. Strains were grouped by phage type (PT), a classification associated with shedding density. We have shown previously that the two most common *E. coli* O157 PTs in Scottish cattle have distinct shedding patterns: PT 21/28 is significantly more likely to be found in supershedding cattle (a supershedder strain), whereas PT 32 is significantly less likely (a non-supershedder strain) (29).

The distribution of PTs among human primary cases (Fig. 1*A*) showed the supershedder strain, PT 21/28, to be more common than expected given its frequency in cattle ($P < 0.001$) and the non-supershedder strain, PT 32, to be less common than expected ($P < 0.001$). These differences allowed us to quantify the relative risk to humans posed by different shedding concentrations. We compared three relationships (*Materials and Methods*), with the best fit to the data being provided by a threshold relationship. This relationship predicted that the distribution of strains among primary human cases was best explained by the frequency of strains in cattle shedding above a threshold of 1,300 cfu/g feces with a 95% confidence interval of 600–2,300 cfu/g feces. This threshold relationship provides a strikingly good fit to the human data ($P > 0.6$), suggesting that high shedding dominates human risk (Fig. 1*A*).

The prediction that high-concentration shedding of the pathogen in cattle dominates human risk is consistent with the observation that, although supershedding is relatively rare, it dominates the contamination of the environment by cattle; in our data, shedding below 10^3 cfu/g feces was found in 86% of samples, but it accounted for less than 1% of the total bacteria shed (Fig. 1*B*).

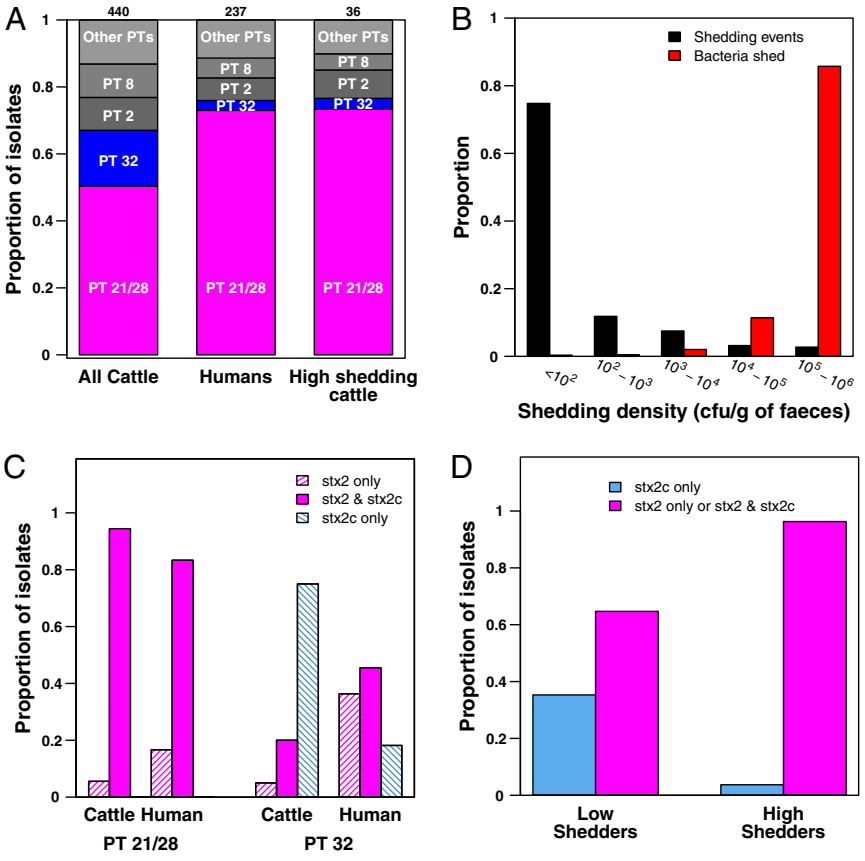

Fig. 1. (*A*) The distribution of PTs among cattle and primary human cases and the link with high-concentration shedding of *E. coli* O157 by cattle. Using the threshold relationship to define high shedding, the distribution of high shedders (>1,300 cfu/g feces) provides a good fit to the distribution of PTs among human cases ($P > 0.6$), suggesting that supershedding dominates human risk. Bootstrapping confirmed the robustness of these results (*Materials and Methods*). (*B*) Most *E. coli* O157 bacteria shed into the environment by cattle arise from infrequent high-concentration shedding events. Shedding density classes are shown by frequency of occurrence (black bars) and contribution to total bacteria shed (red bars). Most shedding is at low densities (black bars), but the majority of bacteria shed arises from high shedding (red bars). (*C*) The distribution of toxin-encoding genes among PTs and in cattle and human isolates. In cattle, PT 21/28 strains were significantly more likely to contain the *stx2* variant than the PT 32 strains ($P < 0.01$). PT 32 strains from humans were more likely ($P = 0.01$) to contain the *stx2* variant than the PT 32 cattle isolates. These observations show an association between the presence of the *stx2* variant and occurrence in human clinical isolates. (*D*) High shedding density and an association with the *stx2* variant. The presence of the *stx2* variant is associated with shedding above the threshold of 10^4 cfu/g feces (odds ratio = 14.2, $P < 0.001$). Strains were grouped into two categories according to whether they were *stx2c* only (blue) or either *stx2* only or *stx2* and *stx2c* (magenta).

Insight into the mechanisms underlying the differences between the PTs was provided by our recent study (35), which showed that the *stx2* variant was more frequent among human PT 32 strains than cattle PT 32 isolates ($P = 0.01$) (Fig. 1*C*). In this study, we additionally show that, among cattle isolates, the *stx2* variant is more common in PT 21/28 than PT 32 ($P < 0.01$) (Fig. 1*C*). These observations show that the presence of the *stx2* variant, whether alone or combined with *stx2c*, is associated with occurrence in human clinical isolates.

We, therefore, examined all PTs in the cattle data for the presence of the *stx2* variant to identify associations with shedding level. Shedding at high concentrations (Fig. 1*D*) was significantly associated with the presence of the *stx2* variant (odds ratio = 14.2, $P < 0.001$ for shedding > 10^4 cfu/g feces; odds ratio = 6.54, $P < 0.001$ for shedding > 1,300 cfu/g feces), indicating that occurrence in humans may arise as a result of high cattle shedding levels associated with this toxin type.

Together, these results support two key conclusions: first, the *stx2* variant is likely to be a critical factor in the shedding phenotype and appearance in human clinical isolates, and second, supershedding of the pathogen by cattle seems to heavily influence risk to humans.

Cattle vaccines that reduce high-concentration shedding should, therefore, be highly effective at reducing human cases. To assess their impact, we simulated the effect of vaccination on shedding frequency and concentration in cattle and predicted the reduction in human cases based on the frequency of shedding above the threshold of 1,300 cfu/g feces—the threshold model of human risk. In our simulations, eliminating just the 12% highest shedding densities produces a 50% drop in the frequency of shedding in cattle (Fig. 2, black line) but an 83% (95% confidence interval = 76–93%) drop in human cases (Fig. 2, red line).

An efficacy of 50% in cattle is consistent with data from vaccine trials, which have been recently summarized in two independent metaanalyses (6, 7). Although the associated reduction in shedding density has typically been assessed for experimental challenge rather than natural infections, Fox et al. (5) report a reduction in frequency of fecal shedding of 46% (consistent with the metaanalyses) and a corresponding reduction in the number of days spent as a high shedder (shedding > 10^3 cfu/g feces) from 0.65 to 0.10 d. This finding is consistent with the effect of vaccination captured in our simulations. Using the threshold relationship directly, this reduction in days spent as a high shedder would predict a reduction in human cases of 85%.

These predictions support our third key conclusion: currently available vaccines that reduce the intensity of shedding should produce substantially greater reductions in human cases than would be predicted from reductions in frequency of shedding in cattle alone.

Discussion

Despite the attention received by supershedding in the cattle reservoir, the relationship with human infection has been unclear. This study shows and quantifies a link between supershedding (high-concentration excretion of *E. coli* O157 in cattle feces) and transmission risk to humans. We use this understanding of the cross-species transmission step to show that the benefit to humans of cattle vaccination should be substantially greater than anticipated from the observed efficacy in cattle. Specifically, we show that vaccines producing a 50% reduction in shedding frequency in cattle (consistent with reported efficacies) could reduce human cases by nearly 85%. We conclude that vaccination of cattle, the major reservoir for *E. coli* O157, could be an especially effective public health control against a serious disease, but studies that do not account for nonlinearities in

POPULATION BIOLOGY

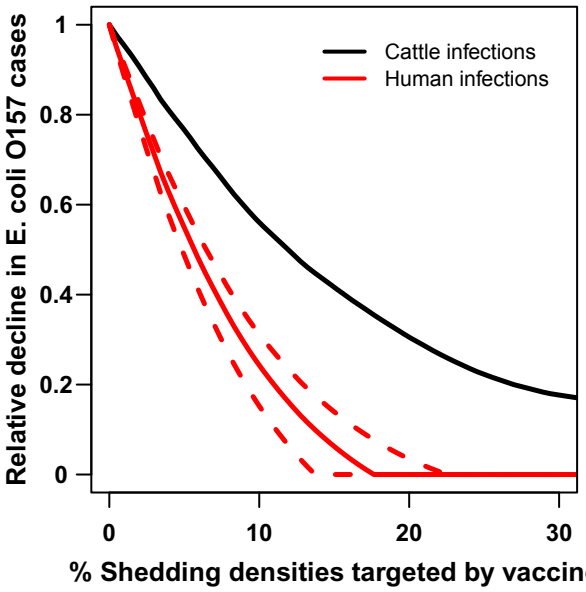

Fig. 2. The predicted impact on the number of human cases of vaccines that reduce high-concentration shedding in cattle. A typical vaccine that reduces high shedding to produce a 50% reduction in shedding frequency in cattle (black line) could result in a reduction in human cases of 83% (red line; 95% confidence limits on the threshold relationship shown as red dashed lines).

cross-species transmission may substantially underestimate the efficacy of interventions against zoonotic pathogens (36).

Zoonotic infections pose particular challenges to public health planning, especially when controls are available in the animal reservoir but the benefit is to the human population. One of the key challenges, which we address here, is predicting how successful controls in animals will be at reducing human illness—a task that is typically hampered by difficulties in mapping transmission across the human–animal interface (18). Our data, which capture both the abundance and strain diversity of *E. coli* O157 in the Scottish human and cattle population across the same time period, are uniquely able to offer insights into the epidemiology of strains that drive human risk.

By examining the drivers of transmission across the species boundary, we offer insights into the selective advantage that Shiga toxin may provide in the cattle reservoir. We show that the Shiga toxin-encoding *stx2* gene variant is likely to be a critical factor behind occurrence in human clinical cases but that this link seems to arise from high levels of pathogen shedding in feces by cattle rather than greater pathogenicity in humans. This observation is medically and epidemiologically important, and it underpins our conclusions about the public health benefits of vaccination. Because the currently available vaccines reduce high-concentration shedding of the pathogen by cattle, which we show to heavily influence transmission across the species boundary, they should be especially effective at reducing the number of human illnesses. Alternatively, if the excess of certain strains in humans had been strongly driven by their pathogenicity in the human host, then vaccines would deliver only proportionate reductions in human cases rather than the very substantial benefit that we predict.

Because the combination of *E. coli* O157 strains present may differ from country to country, the benefits of vaccinating cattle may also vary. However, supershedding is a ubiquitous feature of *E. coli* O157 infection in cattle, and consequently, the capacity for vaccines to deliver substantial reductions in human cases should be robust to changes in geographic setting.

Although *E. coli* O157 is often described as a foodborne pathogen and has been dubbed the burger bug, infections in people arise from a variety of sources: eating contaminated food, drinking from contaminated water supplies, person-to-person transmission, and direct contact with livestock feces (for example, at an open farm or during outdoor pursuits) (37). In Scotland, over one-half of outbreaks are thought to come from environmental exposures (38); in North America, about one-third of cases are attributed to ground (minced) beef, one-third of cases are attributed to produce (e.g., salad vegetables), and one-third of cases are attributed to other sources (2, 39). By tackling *E. coli* O157 at its major source, vaccination could be a very valuable addition to current control measures pre- and postslaughter, because it has the potential to protect people from illness from food and water consumption, exposure at open, petting, or private farms, or living, working in, or visiting rural areas.

A recent study examined the effect of vaccination on human illnesses through ground beef consumption alone (36) and predicted that the reduction in human cases from this source would be less than the observed efficacy in cattle. In contrast, by capturing the nonlinearities across multiple transmission routes, we are able to show that the overall reduction in human cases would be substantially greater (nearly 85%) than the observed efficacy in cattle (~50%). This finding shows the importance of fully accounting for nonlinearities in transmission across the human–animal interface and shows that studies neglecting these phenomena may underestimate the efficacy of interventions.

For zoonoses such as *E. coli* O157, which are do not cause disease in the livestock reservoir, the challenges to the design, evaluation, and delivery of effective interventions are added to by the conflicting responsibilities of veterinary and public health agencies and the lack of economic incentives for food producers.

Estimating the costs and societal benefits of vaccination could provide the basis for an integrated approach by veterinary and public health agencies and help pave the way to cost-sharing solutions. However, there are major challenges in estimating the public health burden of *E. coli* O157, and we are aware that differences in surveillance systems, hospitalization rates, and funding structures for a nation's health system as well as differences in livestock systems will have major impacts on the cost estimates of human disease and conclusions concerning the value of vaccination.

The major costs of *E. coli* O157 infection are caused by hemolytic uremic syndrome (HUS), end stage renal disease, and death (40). In the United States, the total annual societal costs of *E. coli* O157 are estimated at around $600 million (2), with the individual burden and costs per case reaching $6–7 million for individuals progressing to HUS and end stage renal disease or death (40). Societal costs are consequently sensitive to the value given to human life, and a Netherlands-based study that compared economic approaches found US estimates to be nearly double those estimates for HUS in The Netherlands (41). Although direct health costs dominate the estimates, substantial costs arise in other sectors, with the costs of public inquiries and legal action running to millions of dollars (1).

The substantial societal costs, the multiple routes of transmission, and the difficulties for consumers in making well-informed decisions combine to make improved *E. coli* O157 control a public good to be sought (42–45). Cattle vaccination has the potential to provide an important step toward this public good. Estimating the societal costs and benefits of control and determining whether the economic benefits exceed the costs of control will ultimately relate to factors such as vaccine cost, efficacy, and duration of protection, and they are aims that would comprise a substantive separate study.

So far, Canada, where one of the vaccines was developed, is the only country to have granted an *E. coli* O157 vaccine a full commercial license. Uptake, however, is poor, with the vaccine

reaching less than 5% of the market—a likely consequence of the fact that, currently, farmers would bear the cost of vaccination but receive no direct perceived benefit, because the cattle harbor the organism without succumbing to clinical disease. In the United States, two vaccines are available but have not been granted full licenses. Delays in this process arise in part from the separation of licensing bodies for human and veterinary medicines; consequently, licensing a vaccine for use in animals that improves public health would not only save lives but also establish an important public health precedent.

Whether vaccination would ever be widely used in the United Kingdom, North America, and the wider world depends on the costs and benefits of vaccination. We are aware that, currently, the costs would be borne by the farming community with little direct benefit aside from the protection of their immediate families.

The challenges facing *E. coli* O157 control show how an integrated one health approach can help develop effective management strategies for zoonotic disease. An essential step is coordinated data collection in animal and human populations (16, 17). Here, such coordination has provided insights into the dynamics of cross-species transmission and the health benefits to humans of interventions in the animal reservoir. However, despite key studies that show the power of a one health approach (16, 17), greater integration across sectors is needed (14). At the policy level, a coordinated approach would help close the current gap in remit between the separate agencies licensing human and veterinary interventions and provide a starting point for assessing how the costs of interventions might be borne across the multiple stakeholders.

Materials and Methods

Human and Cattle Case Data. The Health Protection Scotland enhanced surveillance system collects data on all culture-positive human cases (46). This analysis included Scotland-acquired primary cases with known PTs identified by the Scottish *E. coli* O157/VTEC Reference Laboratory (SERL). Health Protection Scotland defines primary cases as those symptomatic cases likely to have acquired infection from a food, water, or environmental source of *E. coli* O157 rather than person-to-person spread. Over the study period, from February of 2002 to February of 2004, 237 human cases matched the inclusion criteria. Four isolates not sent to SERL for typing were excluded.

Over the same period, cowpat samples were collected on 481 Scottish cattle farms and examined for *E. coli* O157 presence using immunomagnetic separation (47). Of 12,693 samples, 512 (4%) samples were found positive for *E. coli* O157. SERL phage and toxin typed the isolates. Bacterial counts were obtained for a subset (440) of the positive samples.

PCR to Identify stx2 and stx2c Gene Variants. From 440 cattle isolates with bacterial counts, PCRs to identify *stx2* and *stx2c* gene variants were conducted on a reduced set of 143 isolates selected to avoid duplication of pulsed field gel electrophoresis (PFGE) type/farm combination; 38 of these cattle isolates and 24 human isolates were analyzed by us previously (35). Purified genomic DNA was provided by the Scottish Agricultural College (now SRUC), Inverness via GenePool, University of Edinburgh. Primer sequences were taken from the work by Wang et al. (48), and PCRs were conducted using 2× DreamTaq Green PCR Master Mix (Thermo Scientific Fermentas) according to the manufacturer's instructions; amplified products were separated in 1.3% (g/100ml) agarose gels and imaged using standard procedures.

Relationship Between Human Risk and Shedding Density in Cattle. We compared three alternative relationships between pathogen shedding density in cattle and risk to people as illustrated in the schematic (Fig. 3).

The best-fit relationship was defined as the one that best reproduced the distribution of *E. coli* O157 PTs among the human cases. Underpinning the analysis is that different *E. coli* O157 PTs have different typical shedding distributions (in the text); consequently, different relationships between shedding density and human risk will produce different frequencies of PT occurrence in the human population.

Threshold relationship. Under the threshold relationship, the expected number of human cases of each PT is assumed to be proportional to the number of bacterial counts of that PT exceeding a certain threshold.

Log-linear relationship. Under the log-linear relationship, the contribution to human infection risk from a given shedding density in cattle, denoted *count*, is assumed to be proportional to $(count)^{\alpha}$. Therefore, the expected number of human cases of each PT is proportional to the sum $\Sigma_i(count_i)^{\alpha}$, where i indexes bacterial counts for a given PT.

Logistic dose–response relationship. Under the logistic dose–response relationship, the expected number of human cases of each PT is proportional to the sum $\Sigma_i F(\log(count_i))$, where $F(x)$ is given by

$$F(x) = \frac{1}{1+\exp(-(x-m)/s)};$$

m and s are the midpoint and scale parameters of the logistic function, respectively.

Selecting the best-fitting relationship. Each of the three relationships between risk and shedding density (threshold, logistic, and log-linear) was assessed on its ability to reproduce the proportion of human cases in the PT groupings: PT 21/28, PT 32, PT 2, PT 8, and all other PTs. Maximum likelihood was used to estimate the parameters for each relationship, and the likelihood ratio test or Akaike information criterion (AIC) was used to compare alternative relationships. The robustness of the final relationship was assessed by bootstrapping.

All three risk relationships were compared against a baseline relationship that assumed no relationship between shedding density and risk to humans, and all three relationships provided a better fit to the data at least 99% of the time, showing the baseline model to be inappropriate. The best-fitting logistic dose–response relationship was indistinguishable from the threshold relationship, and therefore, it was not considered further. The threshold relationship was identified as the best relationship in providing a better fit than the log-linear relationship over 96% of the time.

Simulating the Impact of Cattle Vaccination on Human Case Numbers. The impact of vaccination was assessed by combining an epidemiological model of transmission in a cattle herd with the threshold relationship for human risk. The first step was to capture the effect of vaccination on the prevalence and intensity of shedding in a cattle herd. To do this first step, we extended the results of a previous analysis of heterogeneity in cattle-to-cattle *E. coli* O157 transmission (32). This study quantified the relationship between shedding density and cattle-to-cattle infectiousness, thus generating a relationship between the distribution of shedding densities in a cattle herd and the basic reproduction ratio, R_0.

Here, we used this relationship to translate a change in the shedding density distribution (through the prevention of high shedding densities by vaccination) into a reduction in R_0. The corresponding reduction in prevalence in the cattle herd was then simulated using a susceptible–infected–susceptible framework:

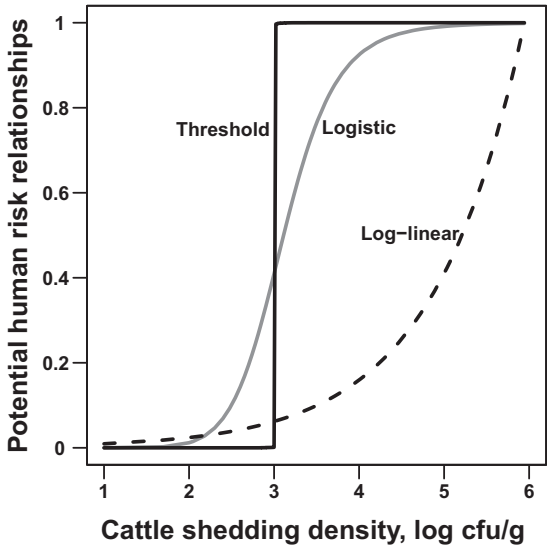

Fig. 3. Schematic of potential relationships between cattle shedding density and human risk. Illustrative curves for the three alternative relationships between shedding density and risk to people are shown: a threshold relationship (black line), a logistic dose–response relationship (gray line), and a log-linear relationship (dashed line).

$$\frac{dI}{dt} = \beta \frac{SI}{N} + eS - \sigma I,$$

where S, I, and N denote the numbers of susceptible and infected cattle and the total herd size, respectively; σ is the recovery rate, e represents infection from external sources (an environmental reservoir plus movement on of infected animals), and β is the mean cattle-to-cattle transmission rate. Parameters were chosen to correspond to a default R_0 of 4.5 (in the absence of vaccination) and an external infection rate of 0.007 as previously estimated for PT 21/28 (32).

Using this model, we simulated the impact of vaccines that prevent high shedding by running scenarios in which we assumed a percentage of the highest shedding densities to be replaced with low shedding densities from the tail of the shedding density distribution. The altered shedding distribution determined the reduction in R_0. The model was used to simulate the reduction in prevalence in the cattle population. The predicted prevalence and the adjusted shedding distributions were substituted into the threshold relationship to predict the corresponding reduction in human infections.

ACKNOWLEDGMENTS. This study was originally funded by a Wellcome Trust International Partnership Research Award in Veterinary Epidemiology (IPRAVE) and the Biotechnology and Biological Sciences Research Council Institute Strategic Programme in Innate Immunity and Endemic Disease at The Roslin Institute (D.L.G. and S.P.M.). We acknowledge additional support from the Food Standards Agency Scotland (L.M. and J.C.L.), the Wellcome Trust (L.M. and D.T.H.), the Biotechnology and Biological Sciences Research Council Institute Strategic Programme on Livestock Viral Diseases at The Pirbright Institute (R.R.), the Scottish Executive Environment and Rural Affairs Department (J.C.L. and G.J.G.), and the Centre for Immunity, Infection and Evolution and Centre of Expertise on Animal Disease Outbreaks (M.E.C.-T.).

1. Pennington H (2010) Escherichia coli O157. *Lancet* 376(9750):1428–1435.
2. Scharff RL (2012) Economic burden from health losses due to foodborne illness in the United States. *J Food Prot* 75(1):123–131.
3. Stevens MP, van Diemen PM, Dziva F, Jones PW, Wallis TS (2002) Options for the control of enterohaemorrhagic *Escherichia coli* in ruminants. *Microbiology* 148(Pt 12): 3767–3778.
4. Thomson DU, et al. (2009) Use of a siderophore receptor and porin proteins-based vaccine to control the burden of Escherichia coli O157:H7 in feedlot cattle. *Foodborne Pathog Dis* 6(7):871–877.
5. Fox JT, et al. (2009) Efficacy of Escherichia coli O157:H7 siderophore receptor/porin proteins-based vaccine in feedlot cattle naturally shedding *E. coli* O157. *Foodborne Pathog Dis* 6(7):893–899.
6. Varela NP, Dick P, Wilson J (2013) Assessing the existing information on the efficacy of bovine vaccination against Escherichia coli O157:H7—a systematic review and meta-analysis. *Zoonoses Public Health* 60(4):253–268.
7. Snedeker KG, Campbell M, Sargeant JM (2012) A systematic review of vaccinations to reduce the shedding of *Escherichia coli* O157 in the faeces of domestic ruminants. *Zoonoses Public Health* 59(2):126–138.
8. McNeilly TN, et al. (2010) Immunization of cattle with a combination of purified intimin-531, EspA and Tir significantly reduces shedding of *Escherichia coli* O157:H7 following oral challenge. *Vaccine* 28(5):1422–1428.
9. US Government Accountability Office (2012) *Preslaughter Interventions Could Reduce E. coli in Cattle* (US Government Accountability Office, Washington DC).
10. Narrod C, Zinsstag J, Tiongco M (2012) A one health framework for estimating the economic costs of zoonotic diseases on society. *EcoHealth* 9(2):150–162.
11. Zinsstag J, Schelling E, Wyss K, Mahamat MB (2005) Potential of cooperation between human and animal health to strengthen health systems. *Lancet* 366(9503):2142–2145.
12. Ayles, A. (2012) Cattle vaccine in Canada could have prevented massive beef recall. Available at www.foodbeat.com/food-news/cattle-vaccine-in-canada-could-have-prevented-massive-beef-recall/. Accessed October 16, 2012.
13. Health Protection Agency (2010) *Review of the Major Outbreak of E. coli O157 in Surrey, 2009. Report of the Independent Investigation Committee* (Health Protection Agency, London).
14. Zinsstag J, et al. (2007) Human benefits of animal interventions for zoonosis control. *Emerg Infect Dis* 13(4):527–531.
15. Bonfoh B, et al. (2012) Representative seroprevalences of brucellosis in humans and livestock in Kyrgyzstan. *EcoHealth* 9(2):132–138.
16. Roth F, et al. (2003) Human health benefits from livestock vaccination for brucellosis: Case study. *Bull World Health Organ* 81(12):867–876.
17. Zinsstag J, et al. (2009) Transmission dynamics and economics of rabies control in dogs and humans in an African city. *Proc Natl Acad Sci USA* 106(35):14996–15001.
18. Lloyd-Smith JO, et al. (2009) Epidemic dynamics at the human-animal interface. *Science* 326(5958):1362–1367.
19. Besser TE, et al. (2007) Greater diversity of Shiga toxin-encoding bacteriophage insertion sites among *Escherichia coli* O157:H7 isolates from cattle than in those from humans. *Appl Environ Microbiol* 73(3):671–679.
20. Bono JL, et al. (2012) Phylogeny of Shiga toxin-producing *Escherichia coli* O157 isolated from cattle and clinically ill humans. *Mol Biol Evol* 29(8):2047–2062.
21. Franz E, et al. (2012) Genetic features differentiating bovine, food, and human isolates of shiga toxin-producing *Escherichia coli* O157 in The Netherlands. *J Clin Microbiol* 50(3):772–780.
22. Persson S, Olsen KEP, Ethelberg S, Scheutz F (2007) Subtyping method for *Escherichia coli* shiga toxin (verocytotoxin) 2 variants and correlations to clinical manifestations. *J Clin Microbiol* 45(6):2020–2024.
23. Jelacic JK, et al. (2003) Shiga toxin-producing *Escherichia coli* in Montana: Bacterial genotypes and clinical profiles. *J Infect Dis* 188(5):719–729.
24. Fuller CA, Pellino CA, Flagler MJ, Strasser JE, Weiss AA (2011) Shiga toxin subtypes display dramatic differences in potency. *Infect Immun* 79(3):1329–1337.
25. Gobert AP, et al. (2007) Shiga toxin produced by enterohemorrhagic Escherichia coli inhibits PI3K/NF-kappaB signaling pathway in globotriaosylceramide-3-negative human intestinal epithelial cells. *J Immunol* 178(12):8168–8174.
26. Robinson CM, Sinclair JF, Smith MJ, O'Brien AD (2006) Shiga toxin of enterohemorrhagic *Escherichia coli* type O157:H7 promotes intestinal colonization. *Proc Natl Acad Sci USA* 103(25):9667–9672.
27. Steinberg KM, Levin BR (2007) Grazing protozoa and the evolution of the Escherichia coli O157:H7 Shiga toxin-encoding prophage. *Proc Biol Sci* 274(1621):1921–1929.
28. Chase-Topping ME, Gally D, Low C, Matthews L, Woolhouse MEJ (2008) Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nat Rev Microbiol* 6(12):904–912.
29. Chase-Topping ME, et al. (2007) Risk factors for the presence of high-level shedders of *Escherichia coli* O157 on Scottish farms. *J Clin Microbiol* 45(5):1594–1603.
30. Stanford K, Agopsowicz CA, McAllister TA (2012) Genetic diversity and antimicrobial resistance among isolates of Escherichia coli O157: H7 from feces and hides of super-shedders and low-shedding pen-mates in two commercial beef feedlots. *BMC Vet Res* 8:178.
31. Robinson SE, Brown PE, Wright EJ, Hart CA, French NP (2009) Quantifying within- and between-animal variation and uncertainty associated with counts of Escherichia coli O157 occurring in naturally infected cattle faeces. *J R Soc Interface* 6(31):169–177.
32. Matthews L, et al. (2009) Exploiting strain diversity to expose transmission heterogeneities and predict the impact of targeting supershedding. *Epidemics* 1(4):221–229.
33. Matthews L, et al. (2006) Super-shedding cattle and the transmission dynamics of Escherichia coli O157. *Epidemiol Infect* 134(1):131–142.
34. Matthews L, et al. (2006) Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. *Proc Natl Acad Sci USA* 103(3):547–552.
35. Xu X, et al. (2012) Lysogeny with Shiga toxin 2-encoding bacteriophages represses type III secretion in enterohemorrhagic Escherichia coli. *PLoS Pathog* 8(5):e1002672.
36. Hurd HS, Malladi S (2012) An outcomes model to evaluate risks and benefits of Escherichia coli vaccination in beef cattle. *Foodborne Pathog Dis* 9(10):952–961.
37. Ogden ID, et al. (2002) Long-term survival of Escherichia coli O157 on pasture following an outbreak associated with sheep at a scout camp. *Lett Appl Microbiol* 34(2): 100–104.
38. Strachan NJC, Dunn GM, Locking ME, Reid TMS, Ogden ID (2006) Escherichia coli O157: Burger bug or environmental pathogen? *Int J Food Microbiol* 112(2):129–137.
39. Greig JD, Ravel A (2009) Analysis of foodborne outbreak data reported internationally for source attribution. *Int J Food Microbiol* 130(2):77–87.
40. Buzby JC, Roberts T (2009) The economics of enteric infections: Human foodborne disease costs. *Gastroenterology* 136(6):1851–1862.
41. Tariq L, Haagsma J, Havelaar A (2011) Cost of illness and disease burden in The Netherlands due to infections with Shiga toxin-producing Escherichia coli O157. *J Food Prot* 74(4):545–552.
42. Unnevehr LJ (2006) *International Association of Agricultural Economists (IAAE).* Available at http://ageconsearch.umn.edu/handle/25733. Accessed October 16, 2012.
43. Richards TJ, Nganje WE, Acharya RN (2009) Public goods, hysteresis, and underinvestment in food safety. *J Agric Res Econ* 34(3):464–482.
44. Fisman DN, Laupland KB (2009) The sounds of silence: Public goods, externalities, and the value of infectious disease control programs. *Can J Infect Dis Med Microbiol* 20(2): 39–41.
45. Jensen HH, Roberts T, Unnevehr L, Hamm S (1995) in The economics of reducing health risk from food. *Proceedings of NE-165 Conference,* ed Caswell JA, Food Policy Marketing Center, Department of Agricultural and Resource Economics, University of Connecticut.
46. Locking ME, et al. (2011) *Escherichia coli* O157 infection and secondary spread, Scotland, 1999-2008. *Emerg Infect Dis* 17(3):524–527.
47. Foster G, et al. (2003) A comparison of two pre-enrichment media prior to immunomagnetic separation for the isolation of E. coli O157 from bovine faeces. *J Appl Microbiol* 95(1):155–159.
48. Wang G, Clark CG, Rodgers FG (2002) Detection in Escherichia coli of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. *J Clin Microbiol* 40(10): 3613–3619.