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Super-Shedding and the Link Between Human Infection and Livestock Carriage of *Escherichia Coli* O157

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Abstract

There is good laboratory and field evidence that some cattle excrete far more *Escherichia coli* O157 than others; these are known as 'super-shedders'. Super-shedding has important consequences for the epidemiology of *E. coli* O157 in cattle - its main reservoir - and for the risk of human infection, particularly via environmental exposure. Ultimately, control measures targeted at super-shedders may prove to be highly effective. At present, there is limited understanding of both the nature of and the determinants of super-shedding. However, super-shedding has been observed to be associated with colonization at the terminal rectum and it may be also more probable with certain pathogen phage types. More generally, epidemiological evidence suggests that super-shedding may turn out to be important in other bacterial and viral infections.

Verocytotoxi-producing *Escherichia coli* (VTEC), such as *E. coli* O157, are an important group of zoonotic pathogens of significant worldwide public health concern. *E. coli* O157 was first recognised as a human pathogen in 1982 when it was detected as the bacteria responsible for food-borne disease outbreaks in North America. Since 1982, infections have been reported in more than 50 countries in every continent other than Antarctica (FIG. 1). Over the past 25 years *E. coli* O157 has become an important cause of severe gastrointestinal illness, often causing bloody diarrhoea that can progress to more serious illness including haemolytic uraemic syndrome (HUS) and death¹. During the 1980's, most reported outbreaks of *E. coli* O157 were food-borne, primarily beef products and unpasteurized milk². Increasingly, however, *E. coli* O157 food-borne outbreaks are being caused by a much wider variety of food products including unpasteurized apple juice, spinach and salami^{3,4}. The probable or confirmed source of contamination for most of these

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foods is cross-contamination. This contamination may originate from faeces and arise from the presence of animals on fields or indirectly via irrigation and/or runoff, or through contamination during packaging and/or food preparation. The highest annual incidences of human infection with *E. coli* O157 during the last two decades have been reported in parts of Canada, the United States, Japan and Scotland^{5,6}. Nationally, the incidence of *E. coli* O157 infection in Scotland is consistently high (1999-2005 the rate per 100,000 population ranged from 3.0 to 5.73)⁷, higher than elsewhere in Britain and abroad (FIG. 1).

Though *E. coli* O157 was initially identified as a food-borne pathogen it is increasingly recognised that environmental exposure can also lead to human infection. Cattle are the main reservoir of *E. coli* O157 in the developed world⁸, though other animals have been shown as possible carriers. Visits to infected farms, contact with animal excreta and recreational use of animal pasture are risk factors contributing to sporadic cases of *E. coli* O157 in humans³. In analyses of the spatial distribution of human cases, incidences of human cases were positively associated with indicators such as livestock density and the ratio of cattle to human population^{9,10,11}. There have been several surveys throughout the world of the proportion of cattle farms with *E. coli* O157 present (TABLE 1). Two of the largest of these were conducted during the past decade in Scotland^{12,13}, both reporting around 20% cattle farms affected.

In this article, we focus on a key observation arising from the Scottish studies, namely that some cattle – referred to as ‘super-shedders’ – excrete far more bacteria than others^{13,14}. Our current understanding of the pathogenesis and epidemiology of super-shedding is summarized in FIG. 2. We review the evidence behind this schema and discuss its implications for the transmission dynamics of *E. coli* O157 in cattle, the risk of human infection (concentrating on environmental rather than food-borne exposure), and the prospects for disease control.

What are super-shedders?

Within the past decade researchers have adopted the terms ‘super-shedding’ and ‘super-spreading’ to describe heterogeneities in the epidemiology of infectious diseases. Although the terms are often used interchangeably we do not regard them as equivalent (Box 1).

Despite an increasing number of published articles on *E. coli* O157 super-shedders, no formal definition has been provided (see Box 1). The results of both field and experimental studies highlight the heterogeneity of *E. coli* O157 carriage and excretion rates. Field studies have demonstrated that the majority (75%) of positive faecal samples contain $<10^2$ colony forming units (CFU) CFU.g⁻¹ faeces of *E. coli* O157¹³. This is above the detection threshold for immunomagnetic separation (IMS) techniques but below the threshold for accurate enumeration¹³. By comparison, a minority of animals may be excreting *E. coli* O157 at levels up to $>10^7$ CFU.g⁻¹ faeces¹³. Such a variation in levels of *E. coli* O157 excretion cannot be explained by a single distribution representing one homogeneous population (FIG 3). The consequences of this are substantial; one study reported that high shedders (there defined as 10^4 CFU.g⁻¹ faeces) made up 9% of a sample of slaughter cattle but were responsible for >96% of all *E. coli* O157 bacteria shed¹⁵. Similar results were

observed in data from the Scottish study¹³ where high shedders (there defined as 3×10^3 CFU.g⁻¹ faeces) comprised 8% of the cattle but 99% of the bacteria shed.

In 2003 it was shown that some *E. coli* O157 infections of cattle colonize the mucosal epithelium at the terminal rectum¹⁶ (FIG. 4). Subsequently, both field and experimental studies have indicated that cattle colonized at this site are associated with the high levels of faecal excretion and longer-duration faecal shedding^{17,18,19}. Non-colonized cattle may only shed bacteria over a few days whereas colonized cattle may shed bacteria for weeks or months^{18,20}. The positive correlation between the concentration of *E. coli* O157 in the faeces and the duration of carriage^{17,20} can be exploited to suggest a working definition of a super-shedder based on a threshold excretion rate of 10^4 CFU.g⁻¹ faeces (Box 1). We propose that super-shedders are the subset of animals that are colonised at the terminal rectum and that replication at this site leads to excretion in the faeces at levels greater than this threshold. By contrast, we suggest that the majority of positive animals shedding at lower levels may be the result of amplification in the faeces during transient passage through the animal host or the result of colonisation with lower level replication at sites other than the terminal rectum. Based on examination of clonal similarity of *E. coli* O157 isolates using PFGE analysis it has been suggested²¹ that *E. coli* O157 in faeces came from two sources: colonized at the terminal rectum or transient in the gastrointestinal tract. This result is confirmed in a study of long-duration *E. coli* O157 colonization¹⁸ where the bacteria were cultured only from the terminal rectum and not from any other location in the gastrointestinal tract.

At present, we have limited knowledge of what leads to rectal colonization and the generation of a super-shedder infection but risk factors may be related to the host (phenotype or genotype), the pathogen (strain) and/or other, 'environment' factors (e.g. route of transmission or exposure dose). One epidemiological study has identified a pathogen-related risk factor for super-shedding¹³. There, super-shedding (defined in terms of faecal bacteria counts – see above) was associated (odds ratio >2) with infections with a particular *E. coli* O157 phage type (PT), PT 21/28. The biological significance of these findings is unclear. It is possible that PT 21/28 may be a marker for some genetic difference or altered gene expression of *E. coli* O157 resulting in that particular subgroup being shed from cattle at higher levels (BOX 2) and therefore more likely to be associated with super-shedding animals.

In addition, a recent study in Canada has also implicated pathogen-related factors. This study revealed strong correlations between the lineage-specific polymorphism assay (LSPA6) genotype and phage type of *E. coli* O157 strains examined²². Previous research had suggested possible genotypic differences may underlie phenotypic differences between LSPA6 lineage I and II genotypes²². These two lineages differ in their frequency of association with human disease. However, further study of *E. coli* O157 strains from other geographic regions is needed to determine whether the association observed is widely applicable²².

The possibilities that super-shedding is also influenced by host genotype or phenotype or environmental factors have yet to be investigated but these may well turn out to be significant.

Super-shedding and within-farm transmission

Natural transmission of *E. coli* O157 between cattle is thought to be largely via the faecal-oral route, although transmission may be indirect via an environmental reservoir²³. It is also possible that wildlife reservoirs and other livestock species play a role as sheep have been reported to excrete concentrations of bacteria comparable with super-shedder cattle²⁴ (see Box 1).

Cattle excreting large numbers of bacteria may be expected to pose a greater risk to other cattle than cattle excreting at low levels²⁵. This is substantiated by data generated from both modelling and experimental studies that have quantified *E. coli* O157 shedding.

Mathematical modelling has suggested that the observed distribution of prevalence of *E. coli* O157 infection at the farm level (most O157-positive farms having low prevalence but a few having very high prevalence) is best explained when a small proportion of cattle are assumed to have much higher transmission rates than the others^{26,14}. Associations have also been observed between the presence of a super-shedder and a high prevalence of low-level shedders on a farm^{17,19,13} – these results are consistent with higher transmission rates where there are super-shedders, although they could simply reflect that (rare) super-shedders are more likely to be found where there are many exposed cattle. However, the same association has also been found in a longitudinal study¹⁹ where the risk of infection was much higher for cattle in a pen with a super-shedder, with the supporting observation that these infections tended to have similar DNA pulsed-field gel electrophoresis (PFGE) type to the super-shedder. In the field, there is often limited PFGE diversity on individual farms²⁷ and on three-quarters of farms with PT 21/28 present no other phage types were found²⁸.

Higher transmission rates on farms with super-shedders could reflect either or both of two effects. First, susceptible cattle are more likely to encounter positive faecal pats and local environmental contamination. Second, when they are exposed they are more likely to be exposed to higher numbers of bacteria. There have been few studies of the effect of dose on *E. coli* O157 infection of cattle, although it is known that oral exposure to <300 CFU can result in infection and that the probability of infection increases with dose²⁹. We do not know the relationship between dose and the production of super-shedders but this could have important effects on the transmission dynamics (BOX 3).

Between-farm transmission

Some studies³⁰ have suggested frequent between-farm transmission on the basis of finding subtypes of *E. coli* O157 that could not be distinguished using macro-restriction fragment analysis of PFGE. Through examination of PFGE types it has been suggested that *E. coli* O157 isolates are frequently transferred over short³¹ as well as long³² and sometimes global distances³³. Data from Scotland show that 15 of 105 PFGE types were found on

more than one farm (out of 91 positive farms), consistent with the results of other studies³⁴ and with the suggestion that between farm transmission does occur.

The routes of between-farm transmissions are not clearly understood. Possibilities include the movement of contaminated faeces or soil via farm personnel or equipment, the movement of contaminated feed³³ or the movement of wildlife (e.g. wild birds) onto farms³⁰. Another potentially important contributor is the movement of cattle between farms. There is much evidence in the literature to support an association between cattle movement and the farm level risk of *E. coli* O157 shedding^{13,35,36,37,38}. Exploratory mathematical models³⁹ have shown that cattle movement can make a significant contribution to the observed prevalence of *E. coli* O157 positive farms but may not be sufficient by itself to maintain *E. coli* O157 in the population⁴⁰. In practice, even closed herds (i.e. those not importing any cattle) are not fully protected against the introduction of *E. coli* O157³⁰.

The role of super-shedders in between-farm transmission and the long term persistence of *E. coli* O157 in the cattle population have yet to be formally investigated. However, since super-shedding is associated with higher on-farm prevalence there will be a greater chance of transmission via movement (and perhaps via other routes too) from a farm with super-shedders (noting that not all cattle are equally likely to be moved to another farm), and presumably a higher chance of establishing infection on an uninfected farm if a super-shedder is imported.

Carriage by cattle and the risk to humans

Environmental exposure is now recognized as an important route for human infection with *E. coli* O157⁴⁰, an observation supported by case-control studies^{41,42,43} and spatial analysis^{9,10,11}. Oral exposure to cattle faeces is significant, but more general environmental exposure is also likely as the bacteria are capable of long-term survival in manure, pasture and soil⁴⁴. A dose-response model has been used to estimate an ID₅₀ for environmental exposure of approximately 10² to 10⁵ bacteria⁴⁵. Exposure doses are typically much lower than this so only a small proportion of exposed individuals will go on to develop an infection⁴⁶. Importantly, there is expected to be a close relationship between numbers of *E. coli* O157 shed by cattle and the proportion of exposed humans infected⁴⁶, implying that environmental contamination by super-shedding cattle results in a much (up to 100-fold or 1000-fold) higher risk.

Sporadic human infections have been related to the presence of cattle, even without knowledge of their status as carriers of *E. coli* O157. Significant clustering of human infection was found in Scotland in regions where the ratio of the cattle population to human population was high¹¹, echoing similar associations reported by studies elsewhere^{9,10}. Recently, it was observed that HUS paediatric incidence was associated with dairy cattle density and the ratio of calves to children <15 years old⁴⁷.

A direct link between cattle and human infections has also been suggested on the basis of subtyping, primarily phage typing and PFGE⁴⁸. In many countries the most common phage types among bovine isolates are also common among human isolates, supporting the idea

that cattle are a principal reservoir⁴⁹. In Scotland, PT 21/28 was by far the most common phage type found, accounting for 50% of the cattle isolates²⁸ and also for 72% of isolates obtained from human infections over the same period (unpublished data). PT 21/28 was also by far the most widespread phage type, found on >50% of all positive farms across Scotland, although with a slight bias toward the north of the country compared to other phage types²⁸.

PT 21/28 is of particular concern because of its association with more severe human morbidity. In Scotland from 1997 to 2001, 61% of HUS cases in individuals less than 16 years old were attributable to infection with PT 21/28⁵⁰. In the UK and Ireland (1997-2001), the risk of developing diarrhoea-associated HUS was significantly higher in children infected with PT 21/28 compared to other phage types⁵⁰. There are two (not mutually exclusive) hypotheses to explain this observation: (i) PT 21/28 is a marker for (so far unidentified) human virulence factors; and (ii) PT 21/28 is typically ingested in higher doses than other phage types. Here, we are particularly concerned with the second hypothesis, which seems plausible given the greater abundance of PT 21/28.

Conclusions

The preceding sections all support the schema shown in Figure 2. It suggests that the epidemiology of *E. coli* O157 may be driven largely by a small number of colonized cattle that for a period are shedding large numbers of bacteria, so called super-shedders (see BOX 1). There is evidence that not all *E. coli* O157 are equally likely to cause super-shedder infections. In Scotland this trait is often associated (though not always) with a particular phage type, PT 21/28 and this has two important implications.

First, there is obviously a need for greater research effort directed at identifying factors that generate a super-shedder. The association with PT 21/28 implicates pathogen factors, and there are a number of candidates for further investigation (see BOX 2). Host genetic factors have yet to be studied; but some aspects of cattle phenotype could be investigated through epidemiological studies of individual-level risk factors for super-shedding. An additional factor that merits attention is the relationship between super-shedding and exposure dose - the form of this relationship has important implications for the population dynamics of super-shedding (see BOX 3).

Second, there are practical implications for the control of *E. coli* O157 and reducing the risk of human infection. To date, consideration of control options for *E. coli* O157 in cattle has not taken super-shedding into account⁵¹. However, the development of a method for identifying super-shedders would allow control strategies to be targeted at removing or eliminating high-level faecal excretion and so to greatly reduce the prevalence of the organism in the host and the risk of human infection⁵². Possible strategies include the detection and removal of super-shedding cattle, testing prior to movement of individual animals, intervention at slaughterhouses and, in the longer term, vaccination to restrict the likelihood of colonization⁵³ or direct treatment of colonized animals⁵². Mathematical models will be useful for exploring the effectiveness of control measures targeted at super-shedders both for reducing bacterial excretion levels in cattle and the risk of infection in humans (both through environmental and food-borne exposure). Ultimately, however, it will

be necessary to implement field trials, and we are optimistic that these will be feasible within the next few years.

In this article we have reviewed evidence surrounding our proposed understanding of the pathogenesis and epidemiology of *E. coli* O157 and its implications for transmission within and among farms, as well as to humans. Central to this article has been the concept of super-shedding cattle. The biology and epidemiology of super-shedding is understood far better for *E. coli* O157 than any other system. Although we have focused on *E. coli* O157 in this article, there is now recognition that super-shedding may be a feature of other bacterial infections, such as *Mycobacterium avium* subsp. *paratuberculosis* (MAP)⁵⁴ and *Salmonella enterica* Typhimurium⁵⁵.

Analysis of outbreak data has suggested that, in general, a minority of individuals are responsible for the majority of infections in a population^{14,56}. If super-shedders are a common phenomenon of most infections, this will have profound implications for our understanding of the epidemiology and control of many infectious diseases. In the case of *E. coli* O157 in cattle, this could potentially lead to a reduction in the risk of human infections. Although we have focussed on environmental exposure, super-shedding does affect carcass contamination and so is likely to be important for food contamination as well⁵⁷.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box 1**What is a super-shedder?****Super-shedders or super-spreaders?**

The terms 'super-shedding' and 'super-spreading' are not always clearly distinguished in the existing literature we therefore recommend the following definitions.

Super-shedder: is a term applied to an individual who for a period yields many more infectious organisms of a particular type than the majority of the same host species. Typically this would mean that many more infectious units are released from a super-shedder. The term is most useful when there is a clear biological basis for the distinction between super-shedders and non-super-shedders (such as host genetic differences, host immune suppression, type differences in the infectious organism, presence or absence of co-infections).

Super-spreader: Super-spreading is a term applied to an individual who has more opportunities to infect other hosts with a given pathogen type than do the majority of individuals of the same host type. Typically this would mean that an individual had many more contacts, where 'contact' is defined by the route of transmission of the pathogen (e.g. direct contact through proximity, physical contact, sexual contact, or indirect contact by contamination of food, being bitten by a large number of vectors etc.)⁵⁸.

Thus defined, super-shedding and super-spreader are independent traits: super-shedding reflects the interaction of the host with the pathogen whereas super-spreading reflects the interaction of the host with other hosts.

***E. coli* O157 super-shedders**

For *E. coli* O157, several working definitions of a super-shedder can be found in the literature. The most basic of these are derived from single direct counts of *E. coli* O157 in faecal samples. Cut-offs for super-shedding have been suggested at counts 10^3 or 10^4 colony forming units (CFU) g^{-1} faeces^{15,17,59,60}, or the simple identification of outlying counts has been used⁶¹.

Such measures are simple to use but do not directly indicate whether an animal is colonized at the terminal rectum, nor do they allow for (possibly substantial) sample-to-sample variation in bacteria counts during the course of an infection. A recent longitudinal study²⁰ using recto-anal mucosal swabs (RAMS) defined a super-shedder on the basis of both mean concentration (10^4 CFU/RAMS) and persistent colonization (4 consecutive positive RAMS, where sampling was carried out twice per week for 14 weeks).

The above definition appears ideal as it encompasses both properties of a super-shedder (high excretion and colonization of the terminal rectum), however, longitudinal examination is not always possible. Hence, for the purpose of identifying super-shedders (for control purposes, for example) we need a suitable working definition that is readily employable in field situations. A recent study²⁰ observed the concentration of *E. coli* O157 in the faeces was positively associated with the estimated duration of culture-

positive status. This is consistent with the findings of a study of cattle at slaughter where shedding $>10^3$ CFU g^{-1} faeces was associated with bacterial carriage close to the terminal rectum whereas shedding $<10^3$ was not¹⁷.

A more formal analysis of *E. coli* O157 faecal counts was performed in a recent paper¹³. Mixture distribution analysis has suggested a cut-off of 3,135 CFU g^{-1} faeces (low and high confidence threshold: 1,658 and 10,395)¹³ (FIG 3). This figure formally confirms the approximated estimates of $>10^3$ to $>10^4$ in the current literature.

On the basis of the available evidence we propose a working definition of an *E. coli* O157 super-shedder as an animal excreting $>10^4$ CFU g^{-1} faeces. Although less stringent than previous recommendations¹⁹, we consider that such high shedding levels are unlikely to occur without colonization. We note, however, that at times during the course of infection or due to sampling variation even colonized animals may excrete at levels below this threshold. In other words, our working definition has high specificity but somewhat lower sensitivity for detecting super-shedders.

It is also possible that wildlife reservoirs and other livestock species play a role as sheep have been reported to excrete concentrations of bacteria comparable with super-shedder cattle²⁴. In a study of sheep at slaughter in Scotland, the counts of *E. coli* O157 in faeces were occasionally $>10^7$ CFU ml^{-1} , suggestive of the existence of other host species as super-shedders of the organism (unpublished data). In addition, a high prevalence of *E. coli* O157 was observed²⁴ in a sheep flock in Scotland with individuals in the flock shedding up to 10^6 CFU g^{-1} .

Box 2**The association between phage type and super-shedding.**

One explanation for an association of phage type (PT) 21/28 with super-shedder infections could be that PT 21/28 is a marker for some key genetic difference or altered gene expression in *E. coli* O157. It has been suggested recently that altered regulation of the type III secretion system (T3S) in PT 21/28 strains compared to other phage type strains may enable the bacteria to colonize and be excreted at higher levels. There is variation in the expression of T3S proteins by different *E. coli* O157 strains that could impact on colonization⁶². It is known that T3S is essential for bovine colonization of cattle at the terminal rectum and therefore the establishment of super-shedders^{16,63,64}. Macro-sequence differences in *E. coli* strains occur as a result of the insertion and deletion of horizontally-acquired genomic 'O' islands (OIs) as well as the acquisition of plasmids⁶⁵. Many of the larger OIs were originally inserted bacteriophage genomes and much of the variation between *E. coli* O157 strains is the result of differences in the OI/phage complement^{66,67,68}. There are different shiga-toxin encoding lambdoid phages and these carry an assortment of accessory genes along with those required for lysogeny/lysis. In addition, their integration sites into the genome can also vary⁶⁹. Phage typing will be responsive to the phage constitution or insertion history as the presence of different phage, or remnants thereof, can provide resistance to infection with another. Hence the association between phage type and super-shedders is likely to represent differences in the OI/phage repertoire of the strain. OIs contain effector proteins that are secreted by the T3S⁷⁰, regulators that control T3S⁷¹ as well as adhesins^{72,73} that may also be directly relevant to bovine colonization. Future work should now correlate the presence of particular OIs with phenotypic and epidemiological data.

Box 3**A simple theoretical framework**

A number of simulation models of the within-herd dynamics of *E. coli* O157 transmission have been developed, but these have not incorporated super-shedders^{74,75,76}. Here, we consider the dynamics of *E. coli* O157 infection in a local, ‘well-mixed’ population of cattle, represented by a modified version of the standard SIS (susceptible-infected-susceptible) model which allows two kinds of infection (*W* for super-shedder; *V* for non-super-shedder) and an environmental reservoir (*E*, measured in ‘units’ of infection).

The model is:

$$\begin{aligned}\frac{dV}{dt} &= \beta_V ES - \gamma_V V \\ \frac{dW}{dt} &= \beta_W ES - \gamma_W W \\ \frac{dE}{dt} &= \lambda_V V + \lambda_W W - \sigma E\end{aligned}$$

where $S=1-V-W$ is the fraction uninfected, $\beta_V E$ and $\beta_W E$ are the per capita rates of infection from the reservoir, γ_V and γ_W are the per capita recovery rates, λ_V and λ_W are the per capita rates infectious units are added to the reservoir, and $1/\sigma$ is the mean survival time of an infectious unit in the environment.

This simple model regards all *E. coli* O157 infections as equivalent, but can readily be adapted to be strain specific. Distinct dynamics for different strains would be represented by variation in any or all of the seven parameters in the model. In particular, for some strains β_W may be very low or even zero, i.e. they rarely or never generate super-shedders. Previously published work¹³ has shown different phage-types to have different propensities to be super-shedders: in this case we might expect the ratio β_W/β_V to be greater for the phage-type 21/28 than other phage-types such as PT32.

Viewing the population as a whole, experimental evidence suggests that $\gamma_V > \gamma_W$, perhaps by an order of magnitude or more. However, the steady state prevalence of super-shedders in Scottish cattle is *lower* than the prevalence of non-super-shedders ($W^* \approx 1\%$ and $V^* \approx 5\%$). This implies that $\beta_V \gg \beta_W$, i.e. that it is very much easier to generate a non-super-shedder than a super-shedder. One possible explanation is that to generate a super-shedder requires an orders of magnitude higher exposure dose. In the case of PT 21/28, for example, a higher prevalence of infection than observed for other PTs, could mean that cattle have an increased likelihood of encountering a higher dose.

The model predicts a steady state level for the environmental reservoir of

$$E^* = (\lambda_V V^* + \lambda_W W^*) / \sigma$$

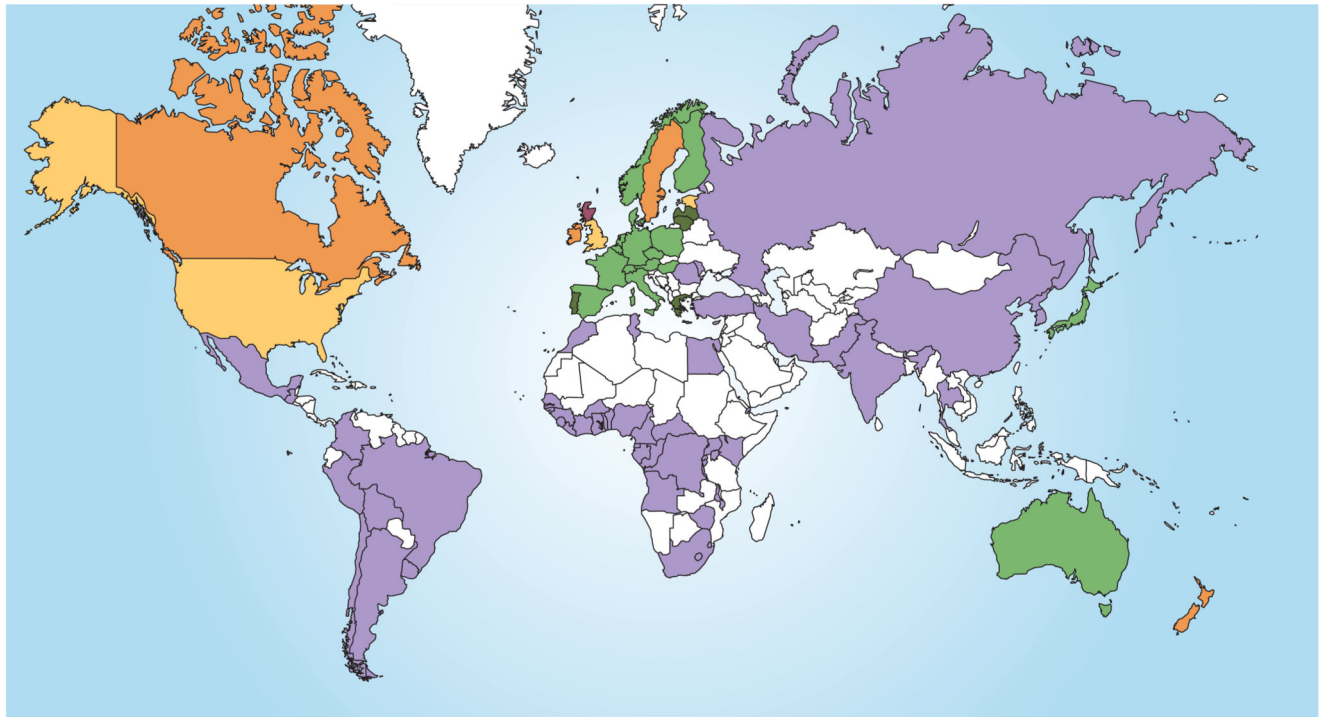
By definition we expect $\lambda_V \ll \lambda_W$, perhaps by two or three orders of magnitude. This difference is much greater than the difference between V^* and W^* , implying that super-shedders make the greatest contribution to the reservoir and so to the risk of subsequent

infection of both cattle and humans. If super-shedders could be rapidly identified and removed (i.e. γ_W becomes very large) then the prevalence of infection will decrease and if

$$\frac{\beta_V \lambda_V}{\sigma \gamma_V} < 1$$

then *E. coli* O157 will be eradicated. However, if σ is small the decline in prevalence will be slow without additional measures directed at the environment reservoir. It is also possible that the reservoir is self-sustaining, either by growth of the bacteria in the environment or if the 'environment' includes alternative host species. Again, this will slow any decrease in prevalence following the removal of super-shedders and could prevent eradication.

The dynamics of *E. coli* O157 infection would be considerably more complex (and the effectiveness of control potentially greater) if there were non-linear effects of exposure dose on the probability of infection, requiring that the term $\beta_W E$ be replaced by a function where incidence is no longer simply proportional to E . If transmission increases more rapidly than linearly with E , then simple models demonstrate that the steady state prevalence responds more readily to reductions in shedding. Such a non-linear effect would arise, for example, if there were a minimum dose needed to generate a super-shedder. The relationship between dose and super-shedding in vivo has yet to be systematically investigated.



Incidence of *E. coli* O157



Figure 1.

Map of the worldwide relative burden of *E. coli* O157 in humans in 2005 per 100,000 population. Crude rates are presented for countries where there are surveillance programs^{77,78,79}, noting that surveillance and detection methods differ and therefore direct comparisons of burden are problematic. Cross hatching represents the detection of *E. coli* O157 in that country but no estimate of incidence rate was available. White represents no data available, noting that infections may have occurred in (some of) these countries, especially those without developed *E. coli* O157 surveillance systems. This figure reflects information in published reports as of August, 2008.

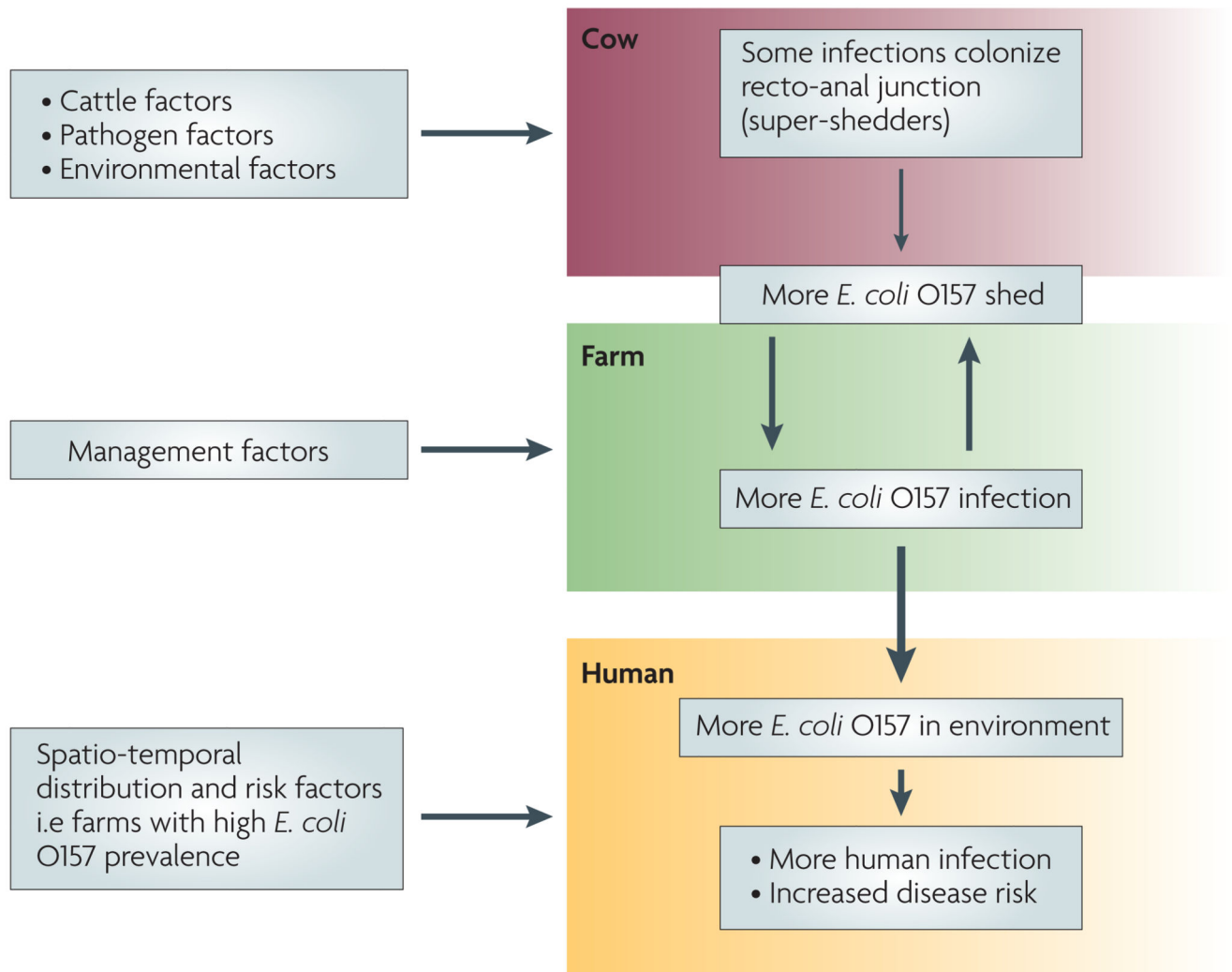


Figure 2. Diagrammatic representation of the chain of events linking super-shedding cattle and the risk of human infection with *E. coli* O157.

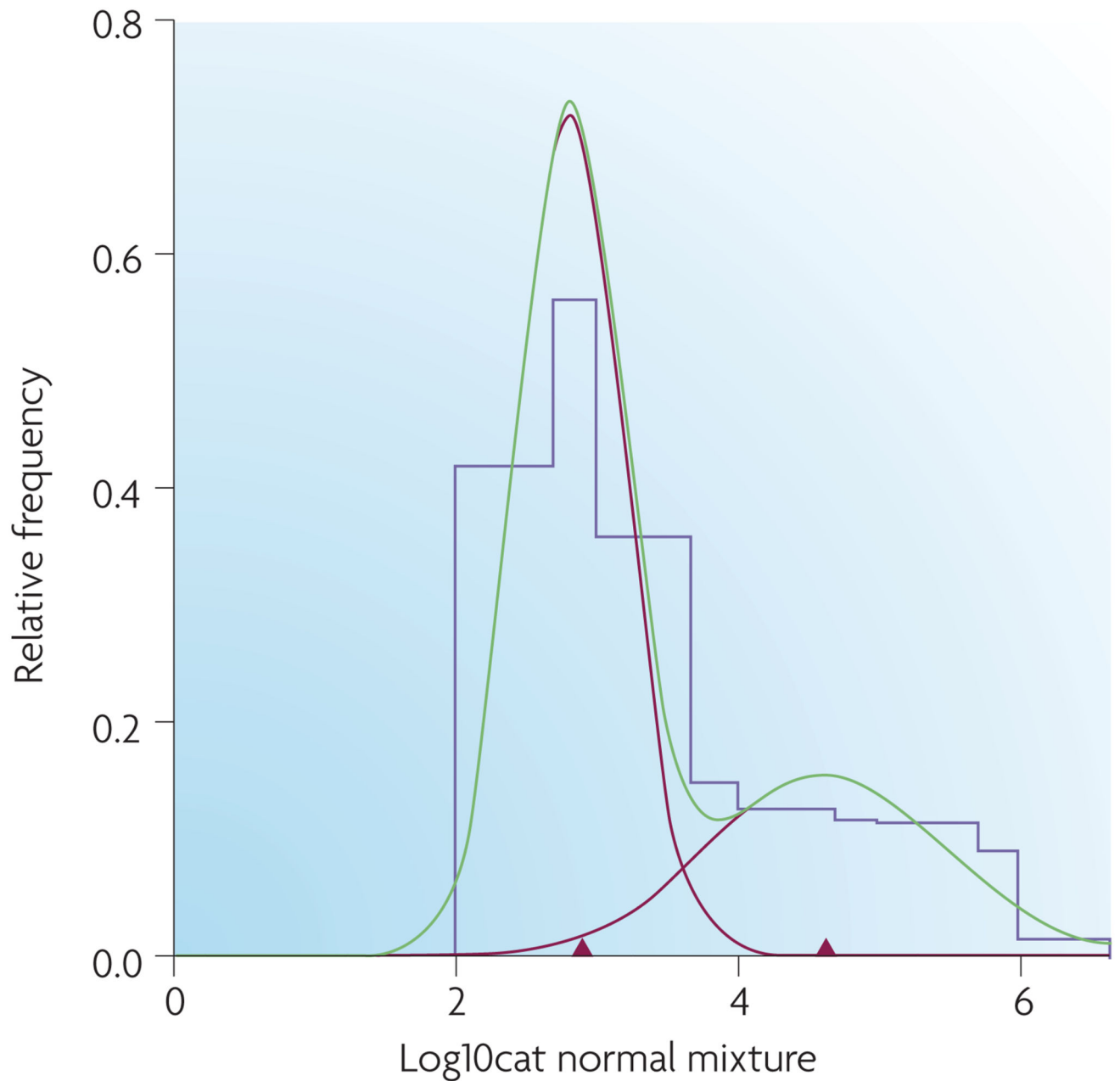


Figure 3.

Results of mixture distribution analysis on a histogram of *E. coli* O157 counts (log transformed) gathered from faecal pats collected during a cross-sectional survey of Scottish store and finishing cattle (2002-2004)¹³. This data was analyzed using MIX (Ichthus Data Systems, Hamilton, Ontario, Canada), a program that analyzes histograms as mixtures of statistical distributions. Parameters of the mixture distribution (mean, standard deviation, proportion) were calculated as maximum likelihood estimates (MLE) using a combination of the Expectation-Maximization (EM) algorithm and Newton-type methods. Final models were fitted assuming two normal components. This figure shows the original histogram of *E.*

coli O157 counts (purple line) fitted with 2 component normal distributions (red lines). The green line is their sum, the mixture distribution, which matches the histogram as closely as possible. The red triangles mark the mean *E. coli* O157 count within each component distribution. The point estimate of threshold for a super-shedder was chosen as the point of overlap of the two unscaled distributions, approximately 3135 C.F.U. g⁻¹; 95% CI for threshold estimate, 1658 and 10395. Note that this does not match the value which might be inferred from this figure, where the components are scaled relative to the sizes of the observed populations. Reproduced with permission from REF 13 © 2007 American society of Microbiology.

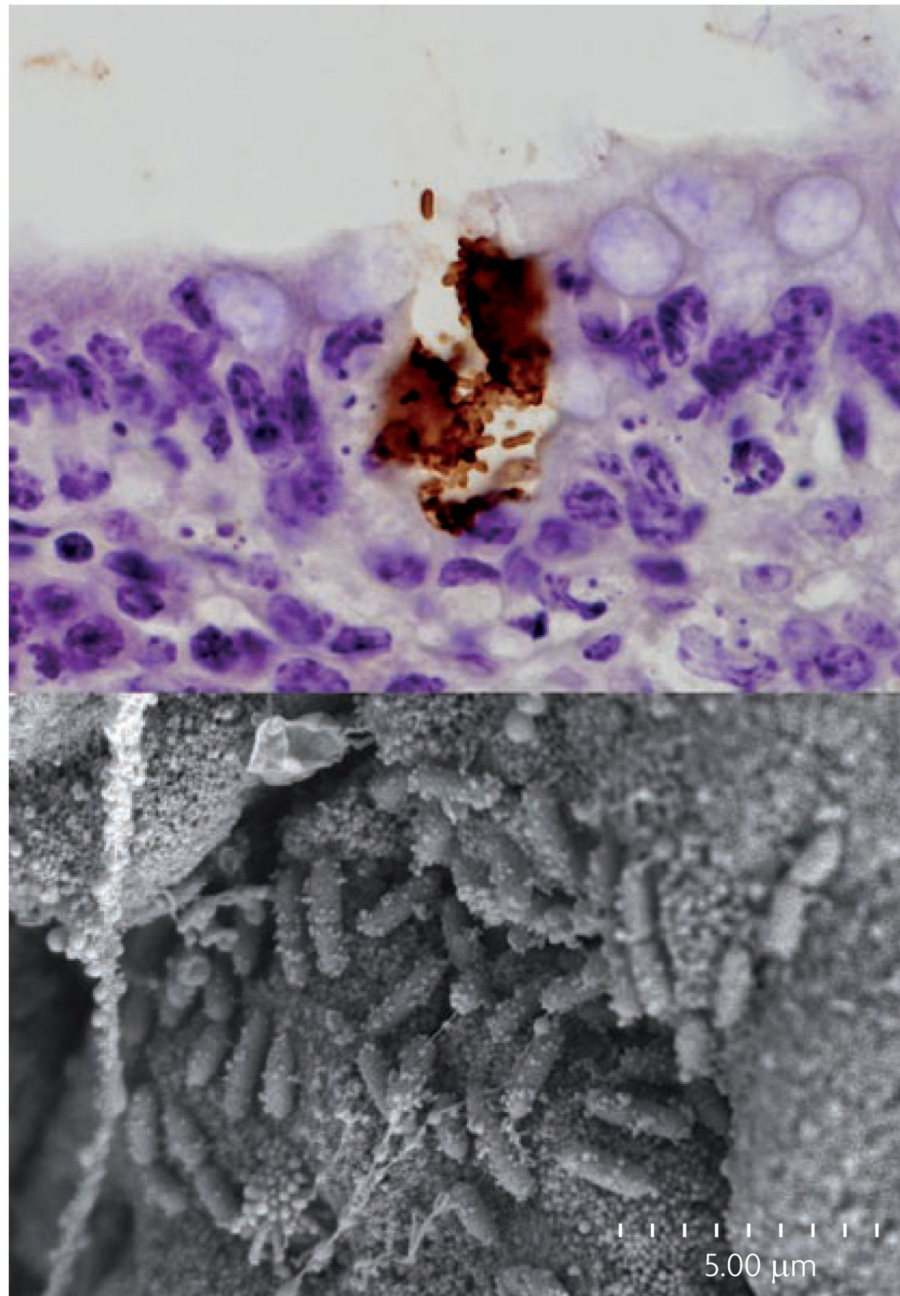


Figure 4. Colonization of cattle terminal rectum by *E. coli* O157. The top panel shows immunoperoxidase staining of a microcolony of *E. coli* O157 at the rectal epithelium of an experimentally-colonized calf. The brown staining is of the surface O157 LPS and shows that the *E. coli* O157 micro-colony has eroded the epithelium (x2000). The bottom panel shows a scanning electron micrograph of a bacterial micro-colony from the terminal rectum

of a calf colonized with *E. coli* O157 (scale indicated). Images provided courtesy of Pablo Nart, University of Edinburgh.

Table 1

Examples of published estimates of *E. coli* O157 farm-level prevalence (proportion of farms tested positive to *E. coli* O157) from different classes of cattle in different countries. True comparisons are difficult as a result of differences in industry and cattle structure between countries as well as differences in study design and laboratory methods. To reduce some of the bias, all studies presented here used the Immunomagnetic bead Separation (IMS) method to detect *E. coli* O157 in the faeces. IMS is a more sensitive technique for generating accurate estimates of *E. coli* O157 prevalence¹².

Country	Cattle Type	N farms	% positive	Ref.
UK:				
England and Wales	Dairy / suckler / fattener	75	38.7	80
Scotland	Store / finishing cattle	952	21.7	12
	Store / finishing cattle	481	18.9	13
Sweden	Dairy cattle	371	8.9	81
Denmark	Dairy cattle	60	16.7	37
Norway	Heifers and milking cows	197	1.0	82
	Dairy cattle	50	0.0	83
Spain	Dairy cattle	124	7.0	84
	Beef cattle	82	1.6	
Netherlands	Dairy cattle	678	7.2	85
	Veal calves	462	9.1	86
	Dairy cattle	10	70	87
Iran	Dairy cattle	26	3.9	88
Canada:				
Saskatchewan	Feedlot cattle	20	60.0	89
Alberta	Feedlot cattle	84	48.0	90
USA:				
Ohio	Dairy cattle	50	8.0	83
Midwest	Ranch and feedlot cattle	29	72.0	91
Tennessee	Dairy cattle	30	26.7	92