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ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

INFORMATION PAPER

The Epidemiology of *E. coli* O157:H7 in cattle and its control, with implications for human infections

The above report on the epidemiology of *E.coli* O157:H7 in cattle and its control with implications for human infections provided by Dr Chris Low (Scottish Agricultural College) is attached for Members information.

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The epidemiology of E. coli O157:H7 in cattle and its control, with implications for human infections (report to ACMSF 1st December 2009).

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Background information

Escherichia coli O157:H7 strains producing the toxin, termed verocytotoxin, are important in causing human disease. The highest rates of *E. coli* O157:H7 human infections, have been reported in parts of Canada, the United States, Japan and Scotland. **The human infection rate in Scotland between 1999-2008 per 100,000 population ranged from 2.9 to 5.6 cases, and was higher than elsewhere in Britain or many countries abroad**.

Although the largest *E. coli* O157:H7 human infection outbreaks have been due to food poisoning, most cases in Scotland are apparently "sporadic", affecting only one person or household. Sporadic infection has been strongly associated with contact with livestock faeces. Cattle are considered the main reservoir of *E. coli* O157:H7 though other animals have also been shown to be possible carriers. **Contact with animal dung and recreational use of animal pasture are known risk factors for human *E. coli* O157:H7 infection**. The incidence of human cases has also been positively associated with livestock density and the ratio of cattle to human population.

Presence of *E. coli* O157:H7 on Scottish farms

There have been several surveys of cattle farms for the presence of *E. coli* O157:H7. Two of the largest surveys were conducted in Scotland, both reporting around 20% cattle farms as positive (that is for at least one collected sample found positive). 952 farms were sampled in the "SEERAD" study and 481 farms in the "IPRAVE" study. Though overall the numbers of farms positive was similar in the two studies individual farms commonly change their status between positive and negative.

Among the *E. coli* O157:H7 isolated in the SEERAD study, 3/1231 (0.2%), 1168/1231 (94.9%) and 60/1231 (4.9%) carried the following verocytotoxins: VT1 only, VT2 only and VT1&VT2 respectively. In the IPRAVE study, 4/508 (0.8%), 455/508 (89.6%) and 45/508 (8.9%) carried VT1 only, VT2 only or VT1&VT2 genes respectively. All were positive for the *eae* gene producing the virulence factor intimin that allows attachment to host cells.

Phage type (PT) 21/28, producing VT2, was the most common type of *E. coli* O157:H7 isolated in both studies (56% of strains in the SEERAD study and 51% of strains in the IPRAVE study). On three-quarters of farms with PT 21/28 present no other phage types were found. The majority of human cases in Scotland during each survey period were also PT 21/28. The proportion of human cases and cattle isolates that were PT 21/28 was much higher than any other phage type

***E. coli* O157:H7 infection in cattle**

The bacterium shows a preference to attach to the terminal portion of the rectum and colonisation of this site results in an unequal distribution of the organism in the faecal stool, i.e. the stool surface is coated with the organism as it is passed. When calves were given the organism by a stomach tube there was evidence of terminal rectal colonisation as early as 3 days later. We have also challenged calves by direct application of the organism to the terminal rectum. This method was effective at establishing colonisation. Tissues from calves shedding *E. coli* O157:H7 have been sampled to examine other sites for colonisation and the contribution of non-rectal colonisation to bacterial shedding levels was minor. Histopathological examination of the terminal rectum from colonised animals showed intimate attachment of the organism and subtle pathological changes, typically alterations of the surface of the rectal cells and a quantifiable increase in inflammatory cells at the site. However, in no animal is it ever possible to detect any clinical sign of rectal colonisation.

Subsequently, both field and experimental studies have shown that **cattle colonised at the rectum are associated with a longer-duration faecal shedding and high levels of faecal excretion**. The finding is also supported by analysis of the gastrointestinal tract of sheep and of slaughter cattle at abattoir. These animals have been termed super-shedders.

What are super-shedder cattle?

Researchers have adopted the terms ‘super-shedding’ and ‘super-spreading’ describing variation in the epidemiology of many infectious diseases. For *E. coli* O157:H7 field studies have shown that the majority (75%) of positive faecal samples contain low numbers of *E. coli* O157:H7 (typically $<10^2$ colony forming units (CFU) $CFU.g^{-1}$ faeces). This level is above the threshold for culture detection but below the threshold for accurate counting. By comparison, a minority of animals may excrete *E. coli* O157:H7 at levels up to $3.6 x 10^7$ $CFU.g^{-1}$ faeces. The consequences of this are substantial; **as high shedders (defined as $\geq 10^4$ $CFU.g^{-1}$ faeces) have made up 9% of a sample of slaughter cattle but were responsible for >96% of all *E. coli* O157:H7 bacteria shed.** Similar results were observed in a study where high shedders (defined as $\geq 3.1 \times 10^3$ $CFU.g^{-1}$ faeces) comprised 8% of the cattle but 99% of the bacteria. Consistent with the experimental studies the non-colonised cattle appear to only shed bacteria over a few days whereas colonised cattle may shed bacteria for a few weeks.

We consider that colonisation will frequently lead to counts in faeces above 3,135 CFU g^{-1} and propose that super-shedders are the subset of animals that are colonised at the terminal rectum where replication at this site leads to high level faecal excretion. It is important to note that super-shedding is not a permanent state but a reflection of the period of rectal colonisation. By contrast, the majority of positive animals shedding lower levels may be the result of amplification in the faeces during transient passage through the host or the result of colonisation with lower level replication at non-rectal sites.

Super-shedding and within-farm transmission

Natural transmission of *E. coli* O157:H7 between cattle is thought to be via the faecal-oral route, although transmission may also be indirect via an environmental reservoir. It is 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 cattle. However, cattle excreting large numbers of bacteria may be expected to pose a greater risk to other cattle than an animal (or even many animals) excreting at low levels. Mathematical modelling has shown that the observed distribution of *E. coli* O157:H7 infection at the farm level (most O157:H7-positive farms having low numbers of samples positive but a few having a very high prevalence) is best explained when a small proportion of cattle are assumed to have much higher transmission rates than the others. Associations have also been observed between the presence of a super-shedder and a high prevalence of low-level shedders on a farm – these results are consistent with higher transmission rates where there are super-shedders, although they could simply reflect that (uncommon) super-shedders are more likely to be found where there are many exposed cattle.

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 exposed then the cattle 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:H7 infection of cattle. We do not know the relationship between dose and the production of super-shedders but this could have important effects on the transmission dynamics of infection.

Between-farm transmission

The routes of between-farm transmissions are not understood. Possibilities include the movement of contaminated faeces or soil on farm personnel or equipment, the movement of contaminated feed or the movement of wildlife (e.g. wild birds) onto farms. Another potentially important contributor is the movement of cattle between farms. Exploratory mathematical models have shown that cattle movement can make a significant contribution to the observed prevalence of *E. coli* O157:H7 positive farms but may not be sufficient by itself to maintain *E. coli* O157:H7 in the population.

Carriage by cattle and the risk to humans

Importantly, there is expected to be a close relationship between numbers of *E. coli* O157:H7 shed by cattle and the proportion of exposed humans infected, 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 relative density of human and cattle, even without knowledge of the status of cattle as carriers of *E. coli* O157:H7.

Control of *E. coli* O157:H7 carriage in cattle

Several options for the control of *E. coli* O157:H7 in cattle have been proposed but in UK none have been demonstrated in the field. Cattle vaccination and challenge trials have been part of our experimental programme and cattle vaccines against *E. coli* O157:H7 have also been developed in USA and Canada.

The "Scottish" vaccine includes a combination of three bacterial antigens; associated with type III secretion (EspA, intimin and Tir are crucial to host cell attachment). Vaccination with these antigens provides significant protection from *E. coli* O157:H7 colonisation and limits shedding levels if an animal does become colonised.

The study of the vaccinated animals' immune responses has shown protection correlates with the generation of specific antibodies present on the rectal mucosa. **We conclude that antigen combinations can be successful in protecting cattle against EHEC O157:H7 colonisation and in reducing shedding levels**. However, there is no knowledge as to whether vaccination administration can be limited to two doses; nor how long the protection will last. Currently, there are no licensed products in Europe though test certificate trials have been carried out in North America.

Additionally, we have studied a novel experimental method, based on the terminal rectal restricted colonisation. In experimentally challenged calves the direct application to the rectal mucosa of an antibacterial agent has greatly reduced *E. coli* O157:H7 shedding levels in the immediate post-treatment period. The direct treatment of the terminal rectum was successful in 35 calves and in only one calf, out of 15 treatments where low level carriage remained, was there evidence of significant non-terminal rectal colonisation.

Control focussing on super-shedding cattle

The apparent importance of super-shedding to the epidemiology of infection in cattle has practical implications for the control of *E. coli* O157:H7 and reducing the risk of human infection. To date, control options for *E. coli* O157:H7 in cattle have not taken super-shedding into account. However, identifying super-shedders would allow control strategies to be targeted at removing or eliminating high-level faecal excretion and so greatly reduce the prevalence of the organism in the host and the risk of human infection. From our work and that of others possible strategies include the detection and removal of super-shedding cattle, testing prior to movement of individual animals, intervention or direct treatment of colonised animals at farm or in slaughterhouses and, in the medium term, vaccination to restrict the likelihood of colonisation.

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