# Qualitative vs quantitative microbiology

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*Escherichia coli* O157 and *Salmonella* are food-borne pathogens of importance to the Australian beef and dairy industries. Cattle are a significant reservoir for both of these pathogens and beef has been the source of food-borne outbreaks of both *E. coli* O157 and *Salmonella*. The presence of pathogens in cattle can lead to contamination of carcasses during slaughter and products produced from these contaminated carcasses pose a risk to consumers.

However, the magnitude of the risk is not clear. Until recently, almost all of the information published on *E. coli* O157 and *Salmonella* in cattle has consisted of only qualitative information i.e. the prevalence of these organisms in cattle. In order to estimate risk, it is important to understand not only how many cattle shed *E. coli* O157 and *Salmonella* but also the number of pathogens shed.

For example, does a group of cattle with only one animal shedding high numbers of *E. coli* O157 or *Salmonella* pose a greater risk than a group where all animals are shedding low numbers? Risk assessments in this area have highlighted the importance of 'high shedders' in determining the final risk [1]. Obtaining quantitative data on the numbers of these pathogens in cattle at the time of slaughter is therefore important.

The observed prevalence of *E. coli* O157 and *Salmonella* in groups of cattle can vary anywhere between 0 and 100%, with individual animals varying in their shedding patterns over time. Methods for enumerating *E. coli* O157 and *Salmonella* in cattle have relied on the Most Probable Number (MPN) technique as it allows detection of low numbers of bacteria in the presence of high numbers of competing background microflora. For example, the population of *E. coli* in cattle faeces is often $10^6$ CFU, whereas *E. coli* O157 and *Salmonella* numbers may be significantly lower. It is therefore difficult to enumerate low numbers of these pathogens (less than 100 CFU/g) by using plating techniques.

Enrichment using MPN methods can detect low numbers of pathogenic bacteria; however, MPN is a statistically based sampling method and is not as accurate as direct plating. The sensitivity of the MPN method can be increased by improving the detection of pathogens using molecular and immunological techniques.

Immunomagnetic separation (IMS) technology has been considered the 'gold standard' for detection of *E. coli* O157 and is becoming established for the detection and isolation of *Salmonella*. Advantages of IMS include not only the specificity and sensitivity of the method, but the target organism is isolated, and can be further characterised by serotyping and for virulence markers. Using molecular techniques such as PCR for confirmation of the presence of an organism in an MPN tube does not provide this information without subsequent isolation of the target organism.

The combination of MPN and IMS has been used by several studies for enumerating *E. coli* O157 in cattle samples [2, 3]. While still not as accurate as direct plating, the method provides valuable information that can be readily incorporated into risk assessments.

A recent study investigating the numbers of *E. coli*, *Salmonella* and *E. coli* O157 in Australian cattle found almost 100% of cattle contained *E. coli* in their faeces with most animals shedding between $10^5$ and $10^7$ CFU/g (Figure 1). *E. coli* O157, a subset of the *E. coli* population, was present in only 13% of the cattle, most of which shed less than 10 MPN/g of faeces [4]. *Salmonella* were present in 7% of the cattle, with the majority of animals shedding less than 10 MPN/g in their faeces [5]. It was concluded from this work that cattle generally shed low numbers of these pathogens, indicating that the risk of carcass contamination in most cases is low. But what happens in those situations where high numbers of pathogens are present in animals?

Some cattle shed high numbers of *E. coli* O157 in their faeces e.g. $10^5$ to $10^6$ per g of faeces [6-8]. These animals have been referred to as 'supershedders' or 'high-shedders' and it is has been proposed

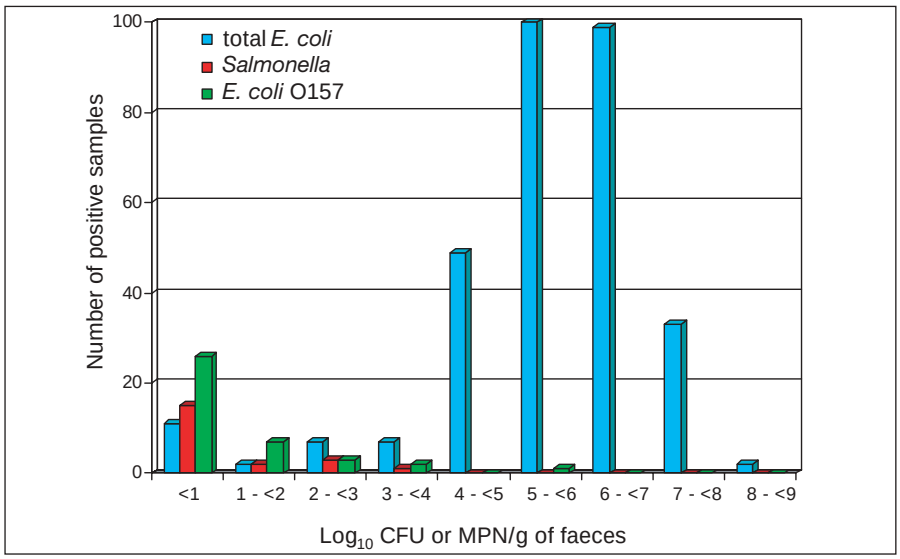


Figure 1. Counts of total *E. coli* (CFU/g), *Salmonella* (MPN/g) and *E. coli* O157 (MPN/g) in 310 cattle faeces collected at slaughter.

that, if such animals are present in a group of cattle being slaughtered, the risk of carcass contamination is increased [6, 9].

When consecutively slaughtered cattle were sampled, high numbers of *E. coli* O157 in faeces and on hides were associated with carcass contamination [Fegan *et al.* manuscript in preparation] (Figure 2). Each animal was tested for the presence of *E. coli* O157 in its faeces and oral cavity and on its hide and carcass prior to chilling. While hardly conclusive, the data suggest that high numbers of pathogens in and on cattle at the time of slaughter are important risk factors for carcass contamination.

While qualitative data are still important, targeted quantitative studies can provide useful information that can allow risk assessors to better model the dynamics of human exposure to food-borne pathogens. Such quantitative assessments can then be used to better inform risk managers and allow them to make more appropriate decisions.

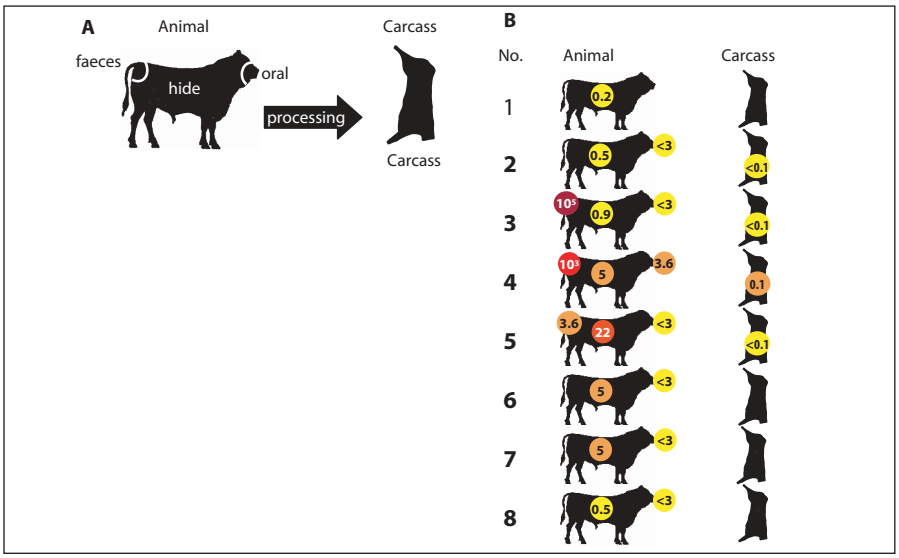


Figure 2. Counts of *E. coli* O157 from different sites of consecutively slaughtered cattle.
A: Sampling sites of each animal included the oral cavity, faeces, hide and the pre-chilled carcass of that animal.
B: Numbers of *E. coli* O157 in eight consecutively slaughtered animals. The positions of the coloured circles indicate the site of the sample and the figures in the circles are the counts of *E. coli* O157 present in that sample. For hides and carcasses, the counts are in MPN/cm$^2$, while for oral cavities and faeces, counts are in MPN/g. The more intensely coloured circles (shades of red) indicate high counts, while paler colours (orange and yellow) indicate lower counts.

The studies presented here are the beginning of such work and highlight some of the dynamics that need to be considered. As methods continue to improve and become more sensitive, quantitative studies will contribute more to our understanding of the ecology of bacterial pathogens in food systems and hopefully lead to safer food.

## References


1. Cassin MH, Lammerding AM, Todd EC, Ross W & McColl RS. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *Int J Food Micro* 1998; 41:21-44.
2. Chapman PA, Cerdan Malo AT, Ellin M, Ashton R & Harkin MA. *Escherichia coli* O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. *Int J Food Micro* 2001; 64:139-150.
3. Barkocy-Gallagher GA, Arthur TM, Rivera-Betancourt M, Nou X, Shackelford SD, Wheeler TL & Koohmaraie M. Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and Salmonella in commercial beef processing plants. *J Food Prot* 2003; 66:1978-1986.
4. Fegan N, Vanderlinde P, Higgs G & Desmarchlier P. The prevalence and concentration of *Escherichia coli* O157 in faeces of cattle from different production systems at slaughter. *J App Micro* 2004; in press.
5. Fegan N, Vanderlinde P, Higgs G & Desmarchelier P. Quantitation and prevalence of *Salmonella* in beef cattle presenting at slaughter. *J App Micro* 2004; in press.
6. Omisakin F, MacRae M, Ogden ID & Strachan NJ. Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *App Env Micro* 2003; 69:2444-2447.
7. Lahti E, Ruoho O, Rantala L, Hanninen ML & Honkanen-Buzalski T. Longitudinal study of *Escherichia coli* O157 in a cattle finishing unit. *App Env Micro* 2003; 69:554-561.
8. Zhao T, Doyle MP, Shere JA & Garber L. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *App Env Micro* 1995; 61:1290-1293.
9. Naylor SW *et al.* Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect Immunity* 2003; 71:1505-1512.