Methodological and Epidemiological Concerns when Comparing Microbial Food Safety Risks from Wildlife, Livestock, and Companion Animals

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Abstract: Outbreaks of foodborne illness associated with the consumption of leafy green produce from California and across the United States have heightened the need to identify vertebrate sources of these microbial hazards. Concern has focused on wildlife species that have direct access to the produce production environment and irrigation water supplies. Recent fecal surveys of California wildlife, feral animals, and livestock and companion animals are allowing regulators to compare the food safety risks of such pathogens as *E. coli* O157:H7 and *Salmonella* from these various animal species. In order to make valid food safety risk comparisons between wildlife, livestock, and companion animals, a variety of methodological and epidemiological issues need to be addressed in order to avoid substantial biases. For example, the amount of feces tested per animal can vary up to a 1000-fold, substantially biasing the probability of testing positive for large fecal contributors (e.g., cattle) compared to smaller wildlife (e.g., deer mice). Many wildlife species intrude and forage as a group in fields of produce, which can lead to in-field defecation, substantially, elevating the risk of microbial contamination compared to many larger animal species that do not have direct access to produce fields due to fencing. This paper highlights the technical challenges of making valid quantitative comparisons of microbial food safety risks from wildlife compared to other domestic animals.

Key Words: cattle, *E. coli* O157:H7, food safety, livestock, public health, *Salmonella*, wildlife

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INTRODUCTION

In order to prevent foodborne outbreaks from produce contaminated with zoonotic enteric pathogens such as *E. coli* O157:H7 and *Salmonella*, we need to identify the biological source(s) of these microbial hazards. Numerous outbreak investigations have indicated that the location of product contamination can occur in the preharvest environment (Jay et al. 2007). Developing targeted and effective preharvest food safety practices is contingent on preventing fecal and microbial contamination of the raw product while it is being grown in the production environment. For example, maintaining the microbiological safety of soil amendments and irrigation water supplies is an important element of these food safety efforts, yet challenges remain given the difficulty of identifying which vertebrate species are the primary sources of enteric pathogens for produce growing throughout the United States.

Plausible biological sources of microbial contamination in the produce production environment are often considered to be humans, wildlife, livestock, and in some cases, companion animals (Gorski et al. 2011, Li et al. 2011, Jay et al. 2007). Prioritizing the risk of foodborne pathogen contamination from different vertebrate species is a problem similar to the difficulty of calculating the environmental loading rate for different vertebrate hosts for waterborne pathogens such as *Crytposporidium parvum* (Atwill et al. 2012). In order to make valid food safety risk comparisons between wildlife, livestock, and companion animals, a variety of methodological and epidemiological issues need to be addressed in order to avoid biases in risk perception. A common approach for prioritizing food safety risks from different host populations is to use the prevalence of fecal shedding of key foodborne pathogens as a proxy for the risk of microbial contamination of produce. For example, do animal populations with a higher prevalence of fecal shedding of *Salmonella* necessarily pose a higher risk to produce compared to animal populations with a lower prevalence of shedding? We have found this approach to be flawed in a variety of scenarios despite its popularity of use among food safety professionals, agriculturalists, and agency staff.

Our objective in the following paper is to highlight several scenarios, some with simulated data, that demonstrate the potential bias in relying solely on the prevalence of fecal shedding of pathogens as a proxy for comparative food safety risk, and we propose that a standardized methodology is required to make valid risk comparisons between wildlife, livestock and companion animals with respect to preharvest microbial food safety.

COMPARING VERTEBRATE SPECIES OF DIFFERENT BODY MASS

The probability of correctly detecting a microbial pathogen for an analytical assay is in part a function of the sensitivity (*Se*) and specificity (*Sp*) of that assay, with *Se* defined as the probability of being test positive given that the pathogen is present in the sample, and *Sp* defined as the probability of being test negative given that the pathogen is not present in the sample. It is well known that *Se* is conditional on the concentration of pathogen in the sample, but in addition, the amount of fecal sample

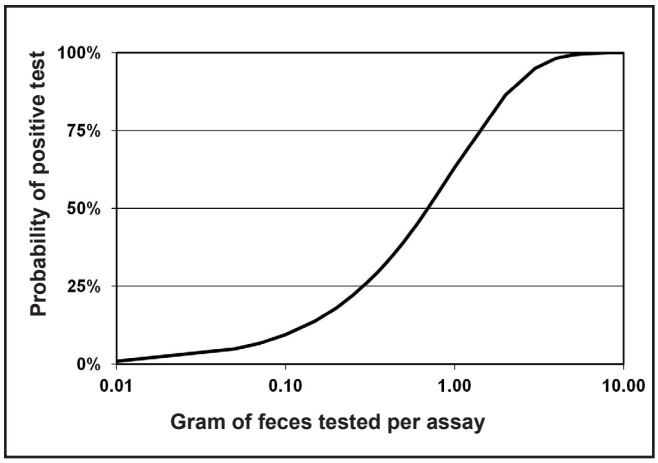

Figure 1. Effect of fecal sample mass on assay sensitivity for a perfect test (*Se* and *Sp* = 100%) and 1 *Salmonella* bacterium per gram.

can also influence the *Se*, especially when the pathogen concentration is less than 100 to 500 microorganisms per gram. As a consequence, biases can occur when comparing the prevalence between vertebrate species if the animal species to be compared have substantially different body mass. This is because the amount of fecal material obtained from animals with a small body mass is typically far less than the amount of fecal material obtained from large animal species. In general, this is the consequence of daily fecal production being a function of body mass and diet. For example, many food animal species produce between 2% to 8% of their body weight in feces (weight wet) per day, depending on their stage of production (see Table 3.2, Atwill et al. 2012), making acquisition of 5 to 100 grams of fecal sample relatively easy for all but the youngest of animals. Similarly, collecting scat samples from large wildlife or feral animals can easily result in fecal samples of 1 to 25 grams (e.g., wild pig, raccoon, coyote). In contrast, non-lethal collection of freshly voided fecal pellets from, for example, a single deer mouse often results in a fecal sample of only 5 to 30 mg. The difference in fecal mass between these groups (small versus large mammals) can vary by as much as a 1,000 times more fecal material. The effect of larger fecal mass on assay sensitivity can be dramatic as shown in Figure 1, resulting in a large influence on the estimated prevalence from field studies and substantially overwhelming an analyst's ability to make valid comparisons between the prevalence of fecal shedding of foodborne pathogens such as *E. coli* O157: H7, *Salmonella*, and *Campylobacter jejuni*.

As a motivating example, assume that different animal species have a 100% prevalence of infection and each population sheds on average one *Salmonella* colony forming unit (cfu) per gram of feces. Using an assay that is 100% sensitive and 100% specific (a perfect test) and assuming that 1) the *Salmonella* bacteria are Poisson distributed within the fecal matrix and 2) inter-animal infection is independent, then random testing of individuals in this population (e.g., cattle, human, feral pig) using a 10 gram fecal sample from each individual will on average result in an estimated prevalence of *Salmonella* of at least 99.9% (which closely matches the truth that all individuals are infected in this theoretical population). The missing 0.1% is due to the random chance that the 10 gram aliquot of feces from an infected individual has no *Salmonella* despite the animal being infected, in effect a false negative for this individual; the bacteria were located in a different part of the stool. In contrast, if we collect a fecal sample from a smaller sized vertebrate that generates scat of 2.5 grams (but has the exact same prevalence of infection of 100% and mean *Salmonella* shedding intensity of 1 cfu/g feces), the estimated prevalence of *Salmonella* infection will be around 92% in these smaller animals (e.g., younger raccoon, opossum). As in the above example, the missing 8% of positive animals is due to the random chance of some of the fecal samples not having any *Salmonella* in the fecal aliquot, regardless that the animal is infected. Despite both populations being 100% infected with *Salmonella*, if we rely solely on the prevalence estimated from a fecal survey, we would conclude that the larger vertebrate (e.g., human, cattle, sheep, feral pig) was a greater risk to food safety compared to the smaller vertebrate (raccoon, opossum). Extending this example further, if one collected 0.5 grams of scat from even smaller vertebrates (e.g., California ground squirrels, black rat) from populations with the same infection levels (prevalence 100%, intensity 1 cfu/g feces), the estimated prevalence of *Salmonella* infection from our scat survey would be 40%. Inferences comparing the prevalences of *Salmonella* shedding between animal species are now heavily biased due to the collection of small scat. Lastly, collecting cloacal swabs from, for example, American house sparrows or collecting fresh fecal pellets from live trapped deer mice, the estimated prevalence of *Salmonella* infection would only be 5% assuming (very optimistically) that one could collect 50 mg of fecal material from each animal (Figure 1). Despite all of these groups of vertebrates being equally 100% infected with *Salmonella*, the impact of different amounts of feces tested per animal substantially biases the estimated prevalence of *Salmonella* infection, with the result being that smaller animals appear to be less infected that larger animals if the fecal mass tested per animal group is different.

Using prevalence as a proxy for food safety risk is in our opinion necessarily biased if the fecal masses being tested are different between vertebrate populations, resulting in an upwardly biased risk profile for vertebrate hosts that generate large fecal samples and a downward bias for vertebrate hosts that generate smaller fecal samples. In other words, large vertebrates are seen as riskier than small vertebrates. In our opinion the literature on microbial food safety risks from different animal species frequently makes this mistake when the prevalence of fecal shedding is compared between large and small animals.

EEFECT OF WILDLIFE AGREGGATING IN GROUPS

Many species of wildlife have direct access to fields of produce such that direct fecal deposition can occur either adjacent to or on top of product intended for human consumption. For example, depending on the style of fencing that the grower or land owner has installed, many smaller mammalian species (mice, voles, rats, ground

squirrels, etc.) are able to pass through the mesh of a fence and either forage and/or establish burrows and nesting sites within or adjacent to fields of produce. In contrast, larger animals may not have such direct access to fields of produce and therefore the risk of fecal contamination of produce is substantially mitigated by the physical distance between fecal loads and actual produce. Produce fields are readily accessible to birds where numerous species either forage along the rows of produce (American crows, starlings, etc.) or use the structure of the produce plant as a perch or nesting site. For example, during certain times of the year avian populations can become quite large, with numbers readily exceeding 100 individuals that forage and/or perch in a field of produce per day.

The risk of pathogen contamination from wildlife that aggregate in fields of produce is substantially larger than is predicted by the prevalence of pathogen shedding in individual animals. For example, assume that 4% of small rodents (e.g., deer mice) are shedding *Salmonella* in their feces at any point in time (point prevalence), a value that we have recently observed in rodents trapped along fields of produce in central coastal California (Vivas et al. 2010). In addition, it is reasonable to assume that an individual rodent defecates at least once (likely much more often) per 24 hours of time while foraging, nesting, or conducting related activities in a field of produce. Under these circumstances, the probability of one or more rodents depositing *Salmonella* in a furrow, bed, or orchard floor rises substantially as the population size increases from 1 to 50 animals (Table 1). For example, the risk of *Salmonella* deposition is 8 times higher when there are 10 rodents in a field compared to one animal; over 15 times higher when 30 rodents have access to the field. Observed population densities of many rodent or avian species per block or field of produce, at least in central coastal California, typically exceeds 10 animals per field, indicating that the aggregate food safety risk from many wildlife species is substantially larger than what is predicted by fecal prevalence studies alone. Depending on the style of fencing and other animal access control measures, livestock and many larger species of wild mammals do not have direct access to fields of produce. In these cases, fecal and associated pathogen contamination must rely on such mechanisms as water and airborne transmission to link fecal loads with more distant produce, or rely on human use of improperly composted manure products to contaminate produce. The validity of food safety risk comparisons between animal species would be improved if the analyst could incorporate a measure of population size and field access in their calculation.

Table 1. The estimated probability of one or more rodents shedding *Salmonella* as a function of rodent density in a produce field, assuming a background level of 4% *Salmonella* infection per rodent on any given day (point prevalence).

Number of rodents in a field of produce	Probability 1 or more rodents shedding *Salmonella* in a produce field[1]
1	4%
5	18%
10	34%
20	56%
30	71%
40	80%
50	87%

[1] Assume infection status independent between rodents, background prevalence is 4%, and probability of shedding one or more rodents estimated using the binomial distribution (e.g., 1-probability of no infected rodents).

ESTIMATING ENVIRONMENTAL LOADING RATES OF FOODBORNE PATHOGENS

A common counter to the arguments presented above is that larger compared to smaller animals produce more fecal mass per day and as a consequence pose a greater risk to produce food safety. In general this is likely to be true: larger amounts of fecal deposition in a field of produce by whatever species poses a greater risk to microbial safety than smaller amounts of fecal contamination, but to accurately make inter-animal comparisons one needs to calculate at a minimum the site-specific environmental loading rate for the pathogen of concern (Atwill et al. 2002, 2012). This parameter takes into account the prevalence and intensity (e.g., cfu/g feces) of pathogen shedding, daily fecal production, and if possible, the analyst should include site-specific population density estimates for the animal species being compared with respect to their access to fields of produce. In this manner, a more comprehensive microbial risk profile is generated for commodities such as produce, drinking or irrigation water, swimming beaches, etc.

When animal populations do not have direct access to the produce commodity of concern and their fecal loads are deposited some distance away, the task of generating inter-animal risk comparisons becomes quite technical and involves complex fate and transport models that attempt to predict the physical movement of microbial pathogens through the environment (e.g., Ferguson and Kay 2012). Given the complexity of fate and transport processes linking pathogen loads to distance fields of produce, generalities are difficult at best, but in many cases the effect of *distance* between fecal loads and commodities of concern, the *dilutional effect* of the transport process (e.g., air, water), and the intervening *time* required to transport the dispersed pathogen load (e.g., inter-storm interval, advection and dispersion in an irrigation reservoir) can substantially reduce the risk of microbial contamination by 100- to more than a 1,000-fold (2 to >3-$\log_{10}$ reduction) compared to the food safety risk of in-field defecation on or adjacent to the product. This argument especially holds for food safety pathogens that cannot replicate outside their hosts, such as viruses and protozoal parasites, but many bacterial pathogens can and will amplify under appropriate environmental conditions. Given the typical scenario of a grower having limited funding for implementing food safety practices, we would argue that growers should minimize animal intrusion and thereby prevent defecation on or near raw produce. When such an approach is practiced in conjunction with reasonable biosecurity measures for irrigation water and soil amendments, this may be a cost-effective approach for promoting produce

food safety when additional off-site pathogen sources are located some distance from fields of produce.

In conclusion, foodborne outbreaks associated with contaminated produce highlight the need to identify the sources of microbial contamination so that effective control measures can be implemented. Relying solely on the prevalence of fecal shedding of key foodborne pathogens to prioritize animal control efforts may result in biased risk perceptions as to which animals are the key species of concern. We recommend a more comprehensive approach that considers not only prevalence, but also incorporates fecal shedding intensity, a measure of animal access to fields of produce, and an appreciation for how food safety risk is substantially elevated when animals aggregate in produce fields compared to being located more remotely. In this manner, we are likely to be more successful in improving food safety through targeted control of species-specific animal intrusion and reduction of in-field defecation.

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