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Prevalence and Serovars of *Salmonella* in the Feces of Free-Ranging White-Tailed Deer (*Odocoileus virginianus*) in Nebraska

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ABSTRACT: To determine the prevalence and serovars of *Salmonella* in free-ranging deer, we cultured feces from white-tailed deer (*Odocoileus virginianus*) harvested by hunters during a regular firearm season in southeastern Nebraska (USA). We recovered *Salmonella* from 5 (1%; 95% confidence interval: 0.37–2.20%) of 500 samples and identified four different *Salmonella enterica* serovars [Litchfield (1), Dessau (1), Infantis (2), and Enteritidis (1)]. Although the prevalence of *Salmonella* in free-ranging deer appears to be low, the serovars recovered are known to be pathogenic to humans and animals.

Key words: Free-ranging deer, *Odocoileus virginianus*, *Salmonella*, white-tailed deer, zoonosis.

Salmonellosis is one of the leading causes of bacterial gastroenteritis in humans and is responsible for over 1.4 million illnesses annually in the USA (Mead et al., 1999). Animals are a primary source of the nontyphoidal *Salmonella* associated with human infections. Although *Salmonella* spp. also cause clinical disease in a variety of animal species, many domestic and wild animals become colonized and shed these bacteria in their feces with no apparent signs of illness (Sanchez et al., 2002). If ingested, either through direct exposure to feces or through fecal contamination of food or water, these bacteria can cause subsequent disease in humans and other animals. Particularly dominant *Salmonella* serovars, some with resistance to multiple antimicrobials, have emerged as a significant public health concern throughout the world, which has led to the development of surveillance and interventions strategies targeted for both human and animal populations (Sanchez et al., 2002).

Wildlife may play a key role in the epidemiology of zoonotic pathogens, but the magnitude of that role depends on many factors (Daszak et al., 2000). Since *Salmonella* can survive well outside the host, and may be shed persistently in the feces of carrier animals (Sanchez et al., 2002), the role of wildlife in the epidemiology of these bacteria may be significant. Free-ranging deer that are infected with *Salmonella* may pose a risk to consumers of venison and individuals with direct or indirect animal contact. In addition, these deer potentially could contaminate recreational or drinking waters. Indeed, wild deer have been implicated as the source of fecal contamination in human infections of *Escherichia coli* O157 associated with the consumption of venison (Keene et al., 1997; Rabatsky-Ehr et al., 2002) and were identified as one of the potential sources of fecal contamination in outbreaks of *E. coli* O157 associated with unpasteurized apple juice (Cody et al., 1999) and contaminated lake water (Feldman et al., 2002). In agricultural areas, deer feces could contaminate produce or livestock feed crops, and wild deer may have direct or indirect contact with food-producing livestock, particularly pastured livestock, resulting in bidirectional transmission. In some segments of the beef cattle industry, for example, deer frequently are seen near cattle (National Animal Health Monitoring System, 1998). The interface between livestock and wildlife and the potential bidirectional flow of disease agents has

been the subject of much recent discussion as emerging sylvatic sources of disease agents has threatened to undermine eradication programs for livestock diseases such as bovine tuberculosis (Bengis et al., 2002).

Clinical salmonellosis has been reported in cervid species, but there are no estimates of the prevalence for *Salmonella* spp. in the feces of free-ranging, apparently healthy deer in the USA. A small outbreak of salmonellosis with significant mortality in captive elk (*Cervus elaphus nelsoni*) in the USA was due to a *Salmonella enterica* serovar Typhimurium DT104 strain, which was shown to be the multiple-drug-resistant epidemic strain common to cattle, sheep, and humans (Foreyt et al., 2001). *Salmonella* also were isolated from clinically ill captive and recently captured red deer calves (*Cervus elaphus*) in New Zealand (McAllum et al., 1978), captive sika deer (*Cervus nippon*) in Japan (Sato et al., 2000), and free-ranging, clinically ill Florida Key deer (*O. v. clavium*) (Nettles et al., 2002). However, previous attempts to identify *Salmonella* spp. in feces from healthy cervid populations have not been successful (Henderson and Hemmingsen, 1983; Pagano et al., 1985; Wahlstrom et al., 2003; Branham et al., 2005). Estimating the prevalence and serovars of *Salmonella* in deer populations will aid in determining the potential impact on domestic and wild animal populations and the risk of human disease from *Salmonella* shed in the feces of deer.

The objective of this study was to determine the prevalence and serovars of *Salmonella* in free-ranging, white-tailed deer harvested by hunters in a grain crop and pasture-based agricultural setting in southeastern Nebraska (USA).

The current study was conducted in conjunction with a study on *E. coli* O157:H7 in deer feces (Renter et al., 2001). Fecal samples from harvested, free-ranging, white-tailed deer were collected and submitted by hunters during a regular firearm season (14–22 November 1998) in a southeastern Nebraska (USA) study area (approximately 25,000 km^2; centered at 40°39′N, 96°39′W). All hunters (approximately 10,000) issued a license for the season and study area were contacted through the mail, provided information on the study, and asked to participate in sample collection. Participating hunters were provided materials and instructions for collecting fecal samples directly from the rectum of harvested deer. Approximately 25% (1,608) of the estimated 6,400 successful hunters submitted samples at one of 28 mandatory area check stations in the study area (Renter et al., 2001). The fecal samples were stored on ice immediately after submission and during transport to the laboratory at Kansas State University (Manhattan, Kansas, USA). To determine *Salmonella* status, a subsample of 500 fecal samples were cultured for *Salmonella*. These samples were the first 500 submitted by hunters and were all collected during the first three days of the hunting season.

One gram of feces was placed in 10 ml of Tetrathionate broth (Difco Laboratories, Cockeysville, Maryland, USA), vortexed, and incubated at 37 C for 48 hr. A 100 µl aliquot of the Tetrathionate/fecal broth was transferred to Rappaport R-10 medium (Difco) and incubated at 37 C for 24 hr. The Tetrathionate/Rappaport was then streaked to XLT4 plates and incubated at 37 C for 24 hr. Colonies with morphologic characteristics consistent with *Salmonella* spp. (black-centered colonies) were screened using lysine agar plates and triple sugar iron slants. *Salmonella*-suspect colonies were characterized further with *Salmonella* O Antiserum Poly A-I and Vi (Difco). Suspect colonies were confirmed as *Salmonella* by serotyping at the Kansas State Veterinary Diagnostic Laboratory (Manhattan, Kansas, USA) and the National Veterinary Service Laboratory (Ames, Iowa, USA). The proportion of positive samples was determined, and the Mid-P method (due to the low prevalence)

was used to calculate a 95% confidence interval (C.I.) using standard statistical software (PEPI, version 4.0, Sagebrush Press, Las Vegas, Nevada, USA).

Approximately 25% (1,608/6,400) of successful hunters in the study area submitted deer fecal samples (Renter et al., 2001), and we analyzed roughly 30% of the samples for this study. All samples were from white-tailed deer and appeared to have normal fecal consistency (fresh soft pellets to loose but thick manure).

We identified *Salmonella* spp. in five of 500 (1.0%; 95% C.I.: 0.37–2.20%) fecal samples. Four different *Salmonella enterica* serovars [Litchfield (1), Dessau (1), Infantis (2), and Enteritidis (1)] were recovered. We believe this is the first report of the prevalence and serovars of *Salmonella* in feces from free-ranging, white-tailed deer. Previous surveys of clinically normal cervid populations failed to identify *Salmonella* spp. in fecal samples from 122 deer farms (3,810 samples) in New Zealand (Henderson and Hemmingsen, 1983), 60 red deer and 13 roe deer in Italy (Pagano et al., 1985), 86 moose, 172 roe deer, and 37 red and fallow deer in Sweden (Wahlstrom et al., 2003), or 26 white-tailed deer harvested by hunters in Texas, USA (Branham et al., 2005). *Salmonella* were detected in 2 (8%) of 26 samples that were collected from the rumen of white-tailed deer harvested on a university ranch in Texas, USA (Branham et al., 2005). The prevalence that we found in deer feces is similar to the overall prevalence in feces (1.4%) reported in a national survey of beef cows in the USA (Dargatz et al., 2000).

Our results indicate an apparently low prevalence of *Salmonella* fecal shedding in free-ranging, white-tailed deer. However, considering the low sensitivity of culturing a single sample of feces for detecting *Salmonella* in other species (Palmer et al., 1985), and that animals can become persistently infected and intermittently shed *Salmonella* spp. in their feces (Sanchez et al., 2002), this point-prevalence estimate might be considered a minimum level of *Salmonella* present in deer. Furthermore, some deer may shed very few organisms in their feces, and we were unable to ensure that samples were handled properly prior to submission, so it is possible that some fecal samples contained *Salmonella* but were not culture positive. More sensitive diagnostic methods such as PCR and/or enhanced culture procedures may be of value for detecting low levels of *Salmonella*. Although commonly used for culturing *Salmonella* from animal feces, the culture methods that we used have not been fully validated for deer feces. Therefore, we cannot estimate the true prevalence based on the apparent prevalence and would not assume that the sensitivity approaches 100%. The true prevalence of infection in animals often is assumed to be higher than estimates based on a fecal culture (Sanchez et al., 2002).

Since our samples were collected over a three-day period, we were not able to examine seasonal differences in fecal shedding. The incidence of human foodborne salmonellosis (Centers for Disease Control and Prevention, 2003a) and prevalence in feedlot cattle feces (Dargatz et al., 2003) are higher in the summer months. The shedding of *Salmonella* in livestock feces also tends to increase during, or following, periods of stress (Sanchez et al., 2002). The deer in our study were sampled during potentially stressful breeding and firearm seasons. However, the fecal samples were collected in the first three days of the hunting season. The deer in this study population also were found to have a low prevalence (<1%) of *E. coli* O157:H7 in their feces (Renter et al., 2001). Because of the low prevalence estimates, we were unable to investigate if an association between *Salmonella* and *E. coli* O157:H7 existed.

All four of the *Salmonella* serovars that we recovered have been associated with clinical disease in humans, and three of the serovars (Litchfield, Infantis, and

Enteritidis) have been reported to cause disease in cattle (Centers for Disease Control and Prevention, 2003b). During the year our deer samples were collected (1998), serovars Infantis and Enteritidis were among the top 20 serovars reported to cause disease in both humans and animals (Centers for Disease Control and Prevention, 1998). For comparison, none of the five most frequently detected serovars in a national survey of beef cows in the USA were among the serovars most likely to cause clinical disease in humans or animals (Dargatz et al., 2000). Typhimurium (McAllum et al., 1978; Sato et al., 2000; Foreyt et al., 2001), Bovis-morbificans (McAllum et al., 1978), and Hartford, Weltevreden, and Kralendyl (Nettles et al., 2002) have been reported to cause disease in deer or elk. Oranienburg was recovered from two rumen samples from deer harvested by hunters on a university ranch (Branham et al., 2005). Since we recovered a very limited number of *Salmonella* isolates, we cannot exclude the possibility that other serovars that are important causes of human and animal disease, such as Typhimurium or Newport, were not, or could not be, present in the feces of apparently healthy, free-ranging, white-tailed deer.

Although prevalence was low, the presence of pathogenic *Salmonella* serovars indicates that feces from apparently healthy deer can be a source of pathogens that infect humans, livestock, and other animals. Bengis and others (2002) discussed how disease issues at the wildlife/livestock interface are frequently bidirectional, which is probable with *Salmonella*. In addition, humans, water, feed, and other factors likely are involved in the micro- and macroepidemiology of *Salmonella* in agroecosystems. Programs that promote livestock biosecurity and wildlife management should account for the fact that wild deer are a potential source of *Salmonella* and that some serovars may be shared among deer, livestock, and humans. People with direct or indirect contact with deer and those consuming, preparing, and processing venison should be made aware of the risks associated with *Salmonella*.

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