Prevalence and Persistence of Pathogens in New York State Road-Kill Disposed of Through Composting: A Literature Review

Prepared by:

Cornell Waste Management Institute
Rice Hall, Ithaca, NY

Mary Schwarz
Ellen Harrison
Jean Bonhotal

For:

New York State Department of Transportation
Elisabeth Kolb, Project Manager

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Executive Summary

Composting is being investigated by New York State Department of Transportation (NYSDOT) as a tool for managing road-killed animals in New York State, particularly white-tailed deer. As part of a project to evaluate the effectiveness of static pile composting to inactivate pathogens in road-killed carcasses, the Cornell Waste Management Institute conducted a literature review and consulted with experts to identify the pathogens that might be present and to assess their sensitivity to inactivation by heating.

The literature reviewed on prevalence suggest that the pathogens expected to be found in white-tailed deer and other wildlife are as follows:

- *Salmonella* – very little to none in deer
- *E. coli* and fecal coliforms – conflicting reports on *E. coli* O157:H7, but other fecal coliform are present and can be a source of human infection
- *Clostridium* – present, especially in the gut and multiply when the animal dies
- *Listeria* – present, but deer are probably not an important source
- *Campylobacter* – very little to none
- *Yersinia* – wild ruminants may be important carriers
- Tularemia (*Francisella tularensis*) and other tick-borne diseases (as well as rabies) are carried by wildlife, but are more important in the handling of the carcasses rather than the composting process
- *Coxiella* – not prevalent
- CWD – present, hard to manage, will most likely not be affected by composting
- *Leptospira* – conflicting reports
- *Cryptosporidia* and *Giardia* – also conflicting reports, but more likely in younger animals
- *Mycobacteria* – present, but probably in less than 5% of the population

The hardiness of these pathogens is summarized in the following table:

	Hardiness Rating		
Pathogen	1	2	3
Salmonella spp.	*		
E. coli and *E. coli* O157:H7	*		
Campylobacter spp.	*		
Yersinia spp.	*		
Listeria spp.	*		
Leptospira spp.		*	
Streptococcus (enterococci)		*	
Clostridium perfringens			*
Mycobacterium			*

A rating of 3 indicates that there is sufficient data to suggest that an organism is capable of surviving when exposed to various stressors, while a rating of 1 would indicate that the organism would not be expected to survive when exposed to stressors (Smith, et al 2005).

Extensive literature review on inactivation of these pathogens suggests that the temperatures reached in static pile composting of road-killed white-tailed deer will be sufficiently high enough to inactivate the pathogens of importance:

- *Salmonella* – unlikely to survive in compost where temperatures exceed 50^{o}C over a period of several days to two weeks. In ground meat, a 1 $\log_{10}$ reduction in bacterial numbers can be obtained after 46 minutes at 55^{o}C, 0.8 minutes at 60^{o}C, and 0.1 minutes at 70^{o}C.
- *E. coli* and fecal coliforms – unlikely to survive in compost where temperatures exceed 50^{o}C over a period of several days to two weeks. In ground meat, a 1 $\log_{10}$ reduction in *E. coli* bacterial numbers can be obtained after 33 minutes at 55^{o}C, 1.2 minutes at 59^{o}C, 0.5 minutes at 60^{o}C, and 0.1 minutes at 70^{o}C.
- *Clostridium* – few studies have been done in compost. In ground meat, a 1 $\log_{10}$ reduction in bacterial numbers can be obtained after 5.2 or 16.9 minutes at 59^{o}C and 41.7 minutes at 70^{o}C.
- *Listeria* – in compost, the rising temperature had little effect on eliminating *Listeria*, but when exposed to the athermic factors (such as alkalinisation of compost to pH 8.8) of composting, it was eliminated. In ground meat, a 1 $\log_{10}$ reduction in bacterial numbers can be obtained after 47 minutes at 55^{o}C and 1.1 minutes at 60^{o}C.
- *Campylobacter* – holding manure at 25^{o}C for 90 days will decrease bacterial numbers to concentrations below detection. In lamb meat, a 1 $\log_{10}$ reduction in bacterial numbers can be obtained after 1.2 minutes at 55^{o}C and 0.3 minutes at 60^{o}C.
- *Yersinia* – holding manure at 25^{o}C for 90 days will decrease bacterial numbers to concentrations below detection. In milk, a 1 $\log_{10}$ reduction in bacterial numbers can be obtained after 0.5 minutes at 60^{o}C.
- *Francisella tularensis* – survived less than 10 minutes in liver and cured ham at 56 and 57^{o}C, respectively.
- *Coxiella* – pasteurization times and temperatures (between 63 and 80^{o}C) needed to kill this organism.
- CWD – extremely high heat needed to inactivate the prion responsible for CWD, will most likely not be affected by composting.
- *Leptospira* – hardiness level two organism. Should be inactivated at the same temperatures as *Streptococcus* spp. In ground beef, a 1 $\log_{10}$ reduction in *Streptococcus faecalis* bacterial numbers can be obtained after 12 minutes at 60^{o}C.
- *Cryptosporidium* and *Giardia* – holding manure at 25^{o}C for 90 days will decrease protozoal numbers to concentrations below detection.
- *Mycobacteria* – hardiness level three organism. Depending on the species, mycobacterium may grow at a wide temperature range from 10 to 65^{o}C, though the optimum growth range for most species is between 29 and 45^{o}C.

In summary, the relative hardiness of the pathogens expected to be found in road-killed deer and other wildlife is *Campylobacter jejuni* < *Yersinia enterocolitica* < *Escherichia coli* < *Listeria monocytogenes* and *Salmonella* spp. < *Streptococcus faecalis* (based on D-values in food from various studies – E&A Environmental Consultants, 2001; Ahmed, et al 1995; Craven and Blankenship, 1983; Lihono, et al 2003; and Price and Tom, 2005).

One of the goals of the literature review was to identify the organisms to be monitored in the field-component of this project. Pilot piles comprised of four deer carcasses embedded in wood chips were established at three NYSDOT facilities around New York State and three replicated research piles were established at Cornell University in Ithaca, NY. At each site, samples of compost are tested periodically

and at the research site, additional testing includes bags of deer intestinal contents that were placed inside the deer carcasses and are retrieved at intervals.

Based on the literature review and consultation, the following pathogens were selected for analysis in both intestinal content bags and compost:

- % solids
- fecal coliform (MPN/g solids)
- *E. coli* (MPN/g solids)
- *Salmonella* spp. (MPN/4 g solids)
- fecal streptococci (MPN/g solids)
- enterococci (MPN/g solids)
- *Mycobacterium Avian paratuberculosis* (MAP) – in intestinal content bags only

Prevalence of Pathogens in Road-Killed White-Tailed Deer and Other Wildlife

Evaluation of the effectiveness of static pile composting to inactivate pathogens in road-killed carcasses first requires identification of the pathogens that might be present. A preliminary meeting held with the faculty at the Cornell School of Veterinary Medicine[1] identified *Clostridium perfringens, Salmonella, Escherichia coli,* fecal or total coliform, *Listeria, Campylobacter, Yesinia, Tularemia, Coxiella,* rabies and chronic wasting disease (CWD) as pathogens/diseases of potential concern in road-killed animals in New York State. Additional communications with experts[2] in wildlife diseases also identified leptospirosis, *Cryptosporidium*, and *Mycobacterium*. A review of the literature for the prevalence of these pathogens in white-tailed deer and other wildlife follows.

Salmonella and *E. coli* are the two most commonly studied bacteria. Mammalian intestines are full of fecal coliform, mostly *E. coli*, but there is particular concern of the pathogenic strain 0157. Most of the literature agrees that *Salmonella* spp. and the *E. coli* O157:H7 are absent from deer. In a study on samples collected from wild red deer, roe deer, moose and reindeer in Norway, *Salmonella* spp. and the potentially human pathogenic verocytotoxic *Escherichia coli* were not isolated (Lillehaug, et al 2005). Weber and Weidt (1986) report 73 roe deer fecal samples were negative for *Salmonella*. No *Salmonella* or *E. coli* O157:H7 were isolated from 450 samples from wild boar, red deer and roe deer in Poland (Koronkiewicz, et al 2004). Henderson and Hemmingsen (1983) report no *Salmonella* spp. were found from 3810 fecal samples of roe deer. They state that the apparent inability of deer to act as carriers [of *Salmonella*] may be because they lack a gall bladder, a site where *Salmonella* spp. can colonize. However, none of these studies were done on white-tailed deer. In one study involving white-tailed deer, Branham, et al (2005) collected samples from deer and other livestock in Texas, in which *Salmonella* spp. were found in the highest quantities in white-tailed deer (7.69%), followed by sheep (7.32%).

In the same study (Branham, et al 2005), *E. coli* O157 was found only in cattle, sheep and water, and only in September (sampling was September through December) most likely due to the well-documented seasonal shedding pattern of these bacteria. In a study on free ranging white-tailed deer in southeastern United States, no *E. coli* O157:H7 were detected in 310 fresh fecal samples collected from the ground. However, when sampled directly from the deer, it was isolated from the feces, but not the meat, of three of 469 (0.64%) deer, but when returning to the same site the following year, it was not found at all in 140 deer (Fisher, et al 2001). The low overall prevalence of *E. coli* O157:H7 and the identification of only one site with positive deer suggest that wild deer are not a major reservoir of *E. coli* O157:H7 in the southeastern United States. Dunn, et al (2004) agree that deer are not a major reservoir, showing only 0.3% prevalence in hunter-harvested deer and 1.8% in captive herds.

Renter, et al (2001), state that even if the presence of *E. coli* O157:H7 in the feces of free-ranging deer is infrequent, any water or food sources contaminated by deer feces should be considered potentially infectious. Their study cultured *E. coli* O157:H7 from 4 (0.25%) of 1,608 hunter-killed white-tailed deer fecal samples in Nebraska. Renter states that the presence of *E. coli* O157:H7 in the feces of free-ranging deer has implications not only for hunters, consumers of venison, and others in contact with deer or deer feces, but also for the development of strategies aimed at reducing and/or controlling this pathogen in water sources and domestic livestock. Others agree although the incidence of prevalence is small; five of 630 (0.79%) over a seven-year period (Rice, et al 2003), five of 212

[1] Edward Dubovi, Patrick McDonough, S.J. Shin, Sue Stehman, Belinda Thompson, and Susan Wade.

[2] Cornell College of Veterinary Medicine: Elizabeth Buckles; NYS Department of Health Services: David Dziewulski; NYS Department of Environmental Conservation: Terry Laibach, Sally Rowland, Alan Woodard; USDA: Richard Chipman, Larry Clark, Pat Millner; EPA: Fran Kremer, Jim Smith; Woods End Research Laboratory: Pam Storms, Will Brinton; Cornell University Department of Natural Resources: Paul Curtis, Gary Goff; Cornell School of Industrial and Labor Relations, Worker Health and Safety Program: Nellie Brown.

(2.4%) of white-tailed deer in Kansas sharing pasture with cattle (Sargeant, et al 1999). Pagano, et al 1985, found no *Salmonella* in fecal samples of wild ruminants and marmots in Italy, but did find antibiotic resistant *E. coli* suggesting they may be important carriers.

According to Rice, et al 1995, it is possible that wild animals sharing the same habitat as cattle may also be colonized by *E. coli* O157 and that interspecies transmission may occur. Deer and cattle fecal samples in an area used by both were collected and cultured for *E. coli* O157. Of the 108 deer samples, two were positive and five of the 191 cattle samples collected were positive. The seven isolates were compared and found to be identical. One case of *E. coli* poisoning from eating venison from white-tailed deer was found in Connecticut. It was found in three packages of meat that had been frozen for 25 days. It was assumed the deer acquired it from cows grazing on dairy farms in Vermont. The authors state that deer are most likely to carry O157 during the time of greatest human exposure, the fall hunting season (Rabatsky-Her, et al 2002). Nine of 11 persons in three households reported symptoms including diarrhea, abdominal cramps, and nausea, from consuming the same venison jerky. *E. coli* O157:H7 was isolated from leftover jerky and one piece of uncooked venison from the same carcass. It was also recovered from the band saw used to cut the deer, and from remnants of the rotting deer skin. *E. coli* O157:H7 was isolated from three (9%) of 32 fecal pellets collected in the surrounding forest one month later, but none was isolated from samples obtained seven months later.

The prevalence of *Clostridium perfringens*, *Listeria*, *Campylobacter* and *Yersinia* in wildlife have all been studied. *Clostridium perfringens* types A and C are found in human and animal feces, soil, green and decaying plant material, sewage, and water (Atwill, 2005). In a study of the causes of morbidity and mortality in farmed white-tailed deer (Haigh, et al 2005), it was found that the following bacterial pathogens were implicated in cases of enteritis and diarrhea in the deer: *E. coli* (n=4), *Clostridium perfringens* (n=2), *Salmonella* spp. (n=2), and *Yersinia* sp. (n=1). Clostridia that survive at low temperatures were isolated from hides, feces and tonsils of deer slaughter stock, making handling of these animals important in slaughter plants to control the spread of *Clostridium* contamination which can cause spoilage of vacuum-packed meats (Broda, et al 2002). *Clostridium botulinum* may be found in the intestinal tract and perhaps other organs of healthy animals. It does no harm there, but, if the animals die, it may multiply and produce toxins in their carcasses.

In a study on 450 animals in Poland, it was confirmed that wild boars, red deer and roe deer were carriers of *Listeria* and *Campylobacter*, but *Yersinia* was isolated only from the feces of wild boar (2.4%) and red deer (18.2%) (Koronkiewicz, et al 2004). In two studies on wildlife, *Listeria monocytogenes* was isolated from deer (7-11%), foxes (14%), badgers (30%), hares (27%) and birds (17%) (Schonberg and Gerigk, 1991). In a study by Weber and Weidt (1986), where the feces of 196 hare and 73 roe deer were studied, *Campylobacter* spp. were isolated from feces from nine hares, but none were isolated from the roe deer. From samples collected from wild red deer, roe deer, moose and reindeer in Norway, *Campylobacter jejuni* was found in one roe deer sample only (Lillehaug, et al 2005). No *Campylobacter* were found in fecal samples from 60 wild red deer, 13 wild roe deer, seven wild chamois, 41 wild alpine marmot and soils mixed with deer feces in Italy, but *Yersinia* was found suggesting wild ruminants may be important carriers of *Yersinia* (Pagano, et al 1985).

This statement is backed up by other studies. In 1984, Henderson obtained fecal samples from farmed and feral deer in New Zealand and isolated 176 strains of *Y. enterocolitica* from 922 samples (isolation rate of 19.1%). Henderson states that this rate exceeds rates described from most other species indicating deer as a major reservoir of *Y. enterocolitica*. In a study done by Shayegani, et al 1983, approximately 1,426 wild animals in New York (most with traumatic injuries) were submitted to the Wildlife Pathology Unit and specimens were collected for identification of pathogens. 133 (9.3%) tested positive for *Yersinia* spp. Distribution across the state positive for *Yersinia* spp. was even, with a range of 4.7 to 12.8% of samples being positive. *Y. enterocolitica* was isolated from the following: 19 of 213

(8.9%) raccoon, 27 of 145 (18.6%) white-tailed deer, nine of 76 (11.8%) gray fox, eight of 59 (13.6%) red fox, and 18 of 196 (10.9%) other mammals.

Tularemia is a very rare disease that is caused by the bacteria *Francisella tularensis*. It is often referred to as rabbit fever or deerfly fever. Humans most commonly contract this disease by handling or eating undercooked wild animal meat that has been infected by disease carrying ticks. Ticks are the most important group of ectoparasites of wild mammals. Soft ticks (family Argaside) feed rapidly, so are rarely collected on their hosts. Hard ticks (Ixodid ticks) require longer blood meals on their hosts. Ixodes scapularis (black-legged tick) is found on white-tailed deer in the eastern United Statesand is responsible for the spread of Lyme disease, human babesiosis, human granulocytic ehrlichiosis, tularemia and spotted fever group Rickettsia. All stages of Ixodes are highly prone to desiccation, and microhabitat contributes greatly to off-host survival (Allan, 2001). The *Ehrlichiae* (small, gram-negative bacteria that primarily invade leucocytes) are grouped according to the type of blood cell most commonly infected. HGE agent (human granulocytic ehrlichiosis) infects granulocytes (the leukocytes involved in the immune defense against pathogens, parasites and allergens), and *Ehrlichia chaffeensis* (the agent involved in human monocytic ehrlichiosis) infects monocytes (leukocytes that develop into macrophages within tissues to ingest bacteria, dead cells, and other debris). Although white-tailed deer harbor a variant strain of the agent of human granulocytic ehrlichiosis not associated with human infection, they are not a reservoir for strains that cause the human disease (Massung, et al 2005). White-tailed deer are, however, natural reservoirs of *E. chaffeensis* and a major host of the Lone Star Tick – the causative agent of human monocytotropic ehrlichiosis (Yabsley, et al 2003).

Coxiella burnetii, the etiologic (the cause or origin of a disease or disorder) agent of Q fever, is a worldwide zoonotic pathogen. Evidence of antibody to *C. burnetii* was reported among various wild-animal species, including coyotes, foxes, rodents, skunks, raccoons, rabbits, deer, and birds. (McQuiston and Childs, 2002). Their literature review suggests that *C. burnetii* is enzootic (a disease that is constantly present in an animal, but usually only affects a small number of animals at any one time) among ruminants and wild animals throughout much of the United States and that there is widespread human exposure to this pathogen. However, sheep and goats appear to be a more important risk for human infection in the United States than cattle or wild animals. A study in Nova Scotia concluded that there is extensive infection of the hare population by *Coxiella burnetti*, with lesser degrees of infection of the moose, raccoon, and deer population (Marrie, et al 1993).

Chronic Wasting Disease (CWD), a fatal brain disease of North American deer and elk, has recently emerged as an important wildlife management issue (Samuel, et al 2002). Despite the lack of evidence that CWD affects humans or livestock, a significant concern has been the perceived risk to humans and livestock. Unfortunately in dealing with CWD, many important biological facts are still unknown and further research will be required to answer these questions.

Destruction of PrP CWD (the prion responsible for CWD) is difficult, and there are few treatments documented to be completely effective; however, high-temperature incineration and alkaline digestion are two such treatments for disposal of CWD-positive carcasses (Fischer, et al 2003). CWD can be transmitted to susceptible animals indirectly, from environments contaminated by excreta or decomposed carcasses (Miller, et al 2004). Under experimental conditions, mule deer (Odocoileus hemionus) became infected in two of three paddocks containing naturally infected deer, in two of three paddocks where infected deer carcasses had decomposed in situ ~1.8 years earlier, and in one of three paddocks where infected deer had last resided 2.2 years earlier. Indirect transmission and environmental persistence of infectious prions will complicate efforts to control CWD and perhaps other animal prion diseases.

In New York State, several CWD-infected deer were found in 2005 in the vicinity of Oneida-Herkimer counties. This area has been designated a containment area and no road-killed deer will be taken from this area or included in composting programs.

There are conflicting reports among the researchers as to whether or not deer are primary carriers of *Leptospira* spp. A total of 403 deer blood specimens (both sexes, ages six months to eight years) were examined for *Brucella abortus* and *Leptospira pomona* in 1958. The results indicated an incidence of 0.25% brucellosis and 1.73% leptospirosis among the deer herds of the southeastern United States (Shotts, et al 1958). Reilly, et al 1962, detected antibodies to antigens of one or more of 10 leptospiral serotypes tested in serum of 23 (22.8%) of 103 deer at the Seneca Ordnance Depot, Seneca County, New York. Roth, et al 1964, report that *Leptospirosis pomona* was isolated in white-tailed deer in IL, MN, WI and LA. Deer can be carriers of leptospires. In 1979, Fleming and Nusbaum found two of 36 (5.5%) samples from white-tailed deer that have no contact with domestic animals were positive for *L. icterohemorrhagiae*, one of 36 (2.8%) were positive for *L. Pomona* and one of 36 (2.8%) were positive for *Toxoplasma*. None were positive for *Brucella*. According to the authors, this survey indicated that deer are not a primary reservoir of *Leptospira* sp., *Brucella* sp. or *Toxoplasma*. A 1986 paper by Ingebrigtsen, et al indicated that tests for antibodies to the etiologic agents of leptospirosis on 628 white-tailed deer produced positive results of only 3%.

White-tailed deer shed *Giardia* sp. cysts and *Cryptosporidium* sp. oocysts in the environment and must be considered potential sources of contamination. However, the incidence decreases in animals greater than six months of age (Rickard, et al 1999). In a study by Perz and LeBlancq 2001, *Cryptosporidium parvum* was found in 22 of 111 wildlife fecal samples collected over a two-year period in lower New York State. They came from 10 of 91 white-tailed deer, three of five chipmunk, one of five raccoon and six of six muskrat. This study provided evidence of *C. parvum* transmission cycles involving deer and other mammalian hosts in lower New York State, affirming the potential role of wildlife species as sources of *Cryptosporidium* in the catchments of public water supplies. In a study in the North Saskatchewan River Basin in Alberta, Canada, *Giardia duodenalis* and *C. parvum*-like oocysts were detected very rarely in wildlife scat samples (66 positive out of 2011 for *Giardia* and 19 out of 2011 for *C. parvum*). The majority of samples containing *Giardia* came from muskrat (78.26%) and beaver (8.68%). *Cryptosporidium* was detected in one deer sample, representing prevalence of 0.15%, and in eight beaver samples, representing a prevalence of 2.4%. However, collection techniques (rectally for the aquatic mammals and on the ground, exposed to elements, for the others) could have caused bias (Heitman, et al 2002). Trout, et al 2003 and 2004 indicate that research suggests deer could be a potential source of infectious cysts of both *C. parvum* and *G. duodenalis* for humans and cattle. The findings of a study that took fecal specimens from 520 dairy calves and 22 coyotes, 82 white-tailed deer and 25 beaver in eastern United States suggest that deer, beaver and cattle could be potential sources of infectious *Giardia* cysts for humans and other animals (Santin-Duran, et al 2004).

Tuberculosis is primarily a respiratory disease and transmission of infection within and between species is mainly by the airborne route. *Mycobacterium bovis*, the cause of bovine-type tuberculosis, has an exceptionally wide host range. Susceptible species include cattle, humans, non-human primates, goats, cats, dogs, pigs, buffalo, badgers, possums, deer and bison (O'Reilly and Daborn, 1995). *Mycobacterium* is a genus of non-sporing, non-motile Gram-positive bacteria. Of the obligate pathogens, the most important include the mammalian tubercle bacilli, which includes *M. bovis* and *M. tuberculosis*. The latter is primarily a human disease, while the former is one of animals, and zoonotic (Clifton-Hadley, 2001). Survival of *M. bovis* outside its host is dependent on ambient environmental conditions: maximum survival occurs in cold, damp conditions, while exposure to direct sunlight under dry conditions lessens its survival. Estimates of distribution of bovine TB in free-ranging white-tailed deer suggest prevalences of less than 5%. The risk to human health is greater for those in close contact with live deer or handling infected carcasses (Clifton-Hadley, 2001).

Conclusion: The literature reviewed here suggest that the pathogens expected to be found in white-tailed deer and other wildlife are as follows:

- *Salmonella* – very little to none
- *E. coli* and fecal coliforms – conflicting reports on *E. coli* O157:H7, but other coliform are present and can be a source of human infection
- *Clostridium* – present, especially in the gut and multiply when the animal dies
- *Listeria* – present, but probably not an important source
- *Campylobacter* – very little to none
- *Yersinia* – wild ruminants may be important carriers
- Tularemia and other tick-borne diseases (as well as rabies) are carried by wildlife, but are more important in the handling of the carcasses rather than the composting process
- *Coxiella* – not prevalent
- CWD – present, hard to manage, will most likely not be affected by composting
- *Leptospira* – conflicting reports
- *Cryptosporidia* and *Giardia* – also conflicting reports, but more likely in younger animals
- *Mycobacteria* – present, but probably in less than 5% of the population

Hardiness and Temperature Sensitivity of Pathogens

The effectiveness of inactivating pathogens through composting is generally assessed by monitoring the reduction in indicator organisms. *Salmonella* and fecal coliform are the usual indicator organisms. These are the organisms that the USEPA requires for evaluation of the hygienic quality of sewage sludges. It is widely recognized that the sensitivity of different pathogenic organisms to heat varies significantly and questions have been raised about the use of the current indicator organisms. Evaluation of the effectiveness of static pile composting to inactivate pathogens in road-killed carcasses requires identification of the pathogens that might be present and analysis of their sensitivity to inactivation by heating. That, combined with time/temperature data from the compost piles, will provide the information needed to assess the hygienic quality of the compost product.

Most of the research done on pathogens in compost has examined *Salmonella*, *E. coli*, and total fecal coliforms. In addition, the compost examined is, most generally, made from manure and farm waste or municipal solid waste. This is because these organisms are responsible for many human gastrointestinal illnesses, and fecal matter is where they are found. According to Deportes, et al 1988, fecal coliforms and fecal streptococci are good candidates for assessing municipal solid waste compost hygienization. Table 1 shows survival and/or inactivation times in compost or manure. Other than in the Shuval, Jiang, and Droffner studies, both *Salmonella* and *E. coli* were either inactivated or undetectable within 24 hours at temperatures greater than 50^{o}C.

Table 1: Survival and/or Inactivation Times of Pathogens in Compost or Manure

Pathogen	Temp (^{o}C)	Time	Source	Comment
S. enteriditis in compost	45	2 days	Lung, et al, 2001	Undetectable
E. coli in compost	45	3 days	Lung, et al, 2001	Undetectable
E. coli in pig manure	50	24 hours	Turner, 2002	Inactivation
E. coli in cattle manure	50	14 days	Jiang, et al, 2003	None detected
E. coli in pig manure	55	2 hours	Turner, 2002	Inactivation
Fecal enterococci in manure	55	2.1 hours	Lund, et al, 1996	4 log reduction
Salmonella in compost	55	80 days	Shuval, et al, 1991	None detected
Fecal coliforms in compost	55	<120 days	Shuval, et al, 1991	5 log reduction
Fecal strep in compost	55	<120 days	Shuval, et al, 1991	4 log reduction
Salmonella in sewage sludge	60	25 min	Mitscherlich and Marth, 1984	Survival time
E. coli in manure compost	60	24 hours	Hess, et al, 2004	Undetectable
Total coliforms in compost	60	24 hours	Hess, et al, 2004	Undetectable
S. typhimurium in food compost	60	9 days	Droffner and Brinton, 1995	Survival time
E. coli in food compost	65	9 days	Droffner and Brinton, 1995	Survival time
M. tuberculosis in biosolids	70	20 min	E&A Environ Consult, 2001	Destruction

In the Shuval study, *Salmonella* was reduced to very low levels in the first few days of composting. *S. enteriditis* and *E. coli* were undetectable in compost after two and three days, respectively, at 45^{o}C (Lung, et al 2001). These data show that *Salmonella* and *E. coli* are unlikely to survive in compost where temperatures exceed 50^{o}C over a period of several days to two weeks. They also show that fecal coliforms and streptococcus may be more resistant to temperature in compost than either *Salmonella* or *E. coli*.

Additional pathogens have been studied to a smaller extent in manure and manure-based composts. Based on actual data plus some data extrapolated from cattle manure environments, holding manure at 25°C for 90 days will decrease pathogens [*Escherichia coli* O157:H7, *Salmonella*, *Campylobacter*, *Yersinia*, *Cryptosporidium*, and *Giardia*] to concentrations below detection (Guan and Holley, 2003). In a study of pathogen survival during mesophilic anaerobic digestion of animal waste, it was found that *Yersinia enterocolitica* was the least resistant of pathogens studied, followed by *Listeria monocytogenes*, *Salmonella typhimurium*, *E. coli* and lastly, *Campylobacter jejuni* (Kearney, et al 1993). Except for *Listeria*, the rising temperature (in short time composting of poultry manure) was sufficient to eliminate the vegetative forms of pathogens investigated (*Salmonella typhimurium, S. pullorum, E. coli, Proteus vulgaris, Pasteurella hamolytica, Past. multocida,* haemolytic *Micrococci*, haemolytic *Streptococci*, and *Listeria monocytogenes Type I*) after exposure to an average 22-hour composting process. *Listeria* was eliminated when exposed to the athermic factors (such as alkalinisation of compost to pH 8.8) of composting. *Clostridium perfringens* was not affected by either temperature or athermic factors (Platz, 1977).

Pathogens of concern in road-killed deer are not necessarily just *E. coli*, *Salmonella* and fecal coliforms. A meeting with Cornell Veterinary College faculty identified, in addition, *Clostridium perfringens*, *Listeria*, *Campylobacter*, *Yersinia*, *Francisella tularensis*, *Coxiella*, rabies and CWD as pathogens that might be associated with deer in New York State. Thermal destruction of these pathogens has been extensively studied in foods. Table 2 gives D-values (minutes needed to get a 1 $\log_{10}$ reduction in bacterial numbers) of these organisms in various foods. It appears from this table that *Campylobacter jejuni* is less resistant to heat than *Yersinia enterocolitica, which* is less resistant than *E. coli*, which is less resistant than *Listeria monocytogenes* and *Salmonella* spp., which are less resistant than either *Streptococcus faecalis* or *Clostridium botulinum. Escherichia coli* is less resistant than *Clostridium perfringens*, but as *C. perfringens* data was not conducted at the same temperature as the others, it is hard to say where it might fit in the whole list. In other words, from least to most hardy: *Campylobacter jejuni* < *Yersinia enterocolitica* < *E. coli* < *Listeria monocytogenes* and *Salmonella* spp. < *Streptococcus faecalis*.

Table 2: D-values of pathogens in various foods

Pathogen	Temp (°C)	Time (min)	Source
Campylobacter jejuni in lamb meat	55	1.2	Price and Tom, 2005
Escherichia coli in turkey	55	8.0	Ahmed, et al, 1995
Escherichia coli in pork sausage	55	8.5	Ahmed, et al, 1995
Escherichia coli in chicken	55	9.3	Ahmed, et al, 1995
Escherichia coli in ground pork	55	33.4	Murphy, et al, 2004
Salmonella in ground pork	55	45.9	Murphy, et al, 2004
Listeria monocytogenes in ground pork	55	47.2	Murphy, et al, 2004
Escherichia coli O157:H7 in ground beef	59	1.2	Doyle and Schoeni, 1984
Clostridium perfringens in ground beef	59	5.2	Roy, et al, 1981
Clostridium perfringens in ground beef	59	16.9	Price and Tom, 2005
Campylobacter jejuni in lamb meat	60	0.3	Price and Tom, 2005
Yersinia enterocolitica in milk	60	0.5	E&A Environ Consultants, 2001
Escherichia coli O157:H7 in ground beef	60	0.5	Ahmed, et al, 1995
Escherichia coli in pork sausage	60	0.5	Ahmed, et al, 1995
Escherichia coli in chicken	60	0.5	Ahmed, et al, 1995
Escherichia coli in turkey	60	0.6	Ahmed, et al, 1995
Salmonella in ground beef	60	0.8	Craven and Blankenship, 1983

Listeria monocytogenes in pork slurry	60	1.1	Lihono, et al, 2003
Salmonella in egg, pH 8.0	60	1.5	Price and Tom, 2005
L. monocytogenes in blue crabmeat	60	1.9	Price and Tom, 2005
L. monocytogenes in lobster	60	2.4	Price and Tom, 2005
L. monocytogenes in mussels	60	5.5	Price and Tom, 2005
Salmonella in egg, pH 5.5	60	9.5	Price and Tom, 2005
Salmonella in pea soup	60	10.0	Price and Tom, 2005
Streptococcus faecalis in fish cakes	60	11.3	Mitscherlich and Marth, 1984
Streptococcus faecalis in tuna pie	60	11.3	Mitscherlich and Marth, 1984
Streptococcus faecalis in chicken a la king	60	12.2	Mitscherlich and Marth, 1984
Streptococcus faecalis in ground beef	60	12.2	Mitscherlich and Marth, 1984
Streptococcus faecalis in fish sticks	60	15.7	Mitscherlich and Marth, 1984
Escherichia coli in ground pork	70	0.1	Murphy, et al, 2004
Salmonella in ground pork	70	0.1	Murphy, et al, 2004
Clostridium botulinum in ground turkey	70	41.7	Juneja, et al, 1995

The pathogens of concern in road-killed deer that do not appear in Table 2 (*Francisella tularensis,* and *Coxiella burnetti*) have been studied. *Francisella tularensis* survived less than 10 minutes in liver and cured ham at 56 and 57^{o}C, respectively. *Coxiella burnetti* is one of the organisms that have been used as an indicator of successful pasteurization of milk. The extent of the pasteurization treatment required is determined by the heat resistance of the most heat-resistant enzyme or microorganism in the food (University of Guelph, 2005) indicating the need for pasteurization times and temperatures (between 63 and 80^{o}C) to kill this organism. Most of the pathogens found in road-killed deer appear to be fairly easily killed by temperatures above 60^{o}C, so in order to assess the effectiveness of destruction of pathogens due to composting, it was decided that indicator organisms should be used.

In food studies, indicator organisms have been generally *Escherichia coli*, *Salmonella, Aeromonas*, *Listeria* and *Yersinia* species (Simpson, et al 1994). But, as seen above, these are not necessarily the most heat resistant. *Streptococcus faecium* is a thermoduric enterococcus microorganism that is most likely to survive the mild pasteurization heat treatment given to some foods, and to withstand the presence of salt and nitrite at normal usage levels (Simpson, et al 1994). Lund, et al 1996, state that data indicate that fecal enterococci measurements give a good indication of inactivation of enterovirus and other more heat sensitive viruses, especially under thermophilic conditions. Due to the high thermal resistance, ability to grow at a wide range of temperatures in the presence of salt and in low pH values, *Enterococcus faecium* has been frequently considered as a reference microorganism for thermal treatments to be applied in pasteurized meals or “sous vide” (a method of cooking that is intended to maintain the integrity of the ingredients by cooking it for many hours at relatively low temperatures – 60^{o}C) type foods (Martinez, et al 2003). *E. faecium* is not expected to be found in deer, but other enterococci are. Based on this, enterococci appear to be a good indicator of pathogen destruction in deer compost piles.

Smith, et al 2005 describe the hardiness of bacteria as the relative ability of the organism to survive environmental stress and/or treatment processes. A rating of three indicates that there is sufficient data to suggest that the organism is capable of surviving when exposed to various stressors, while a rating of one would indicate that the organism would not be expected to survive when exposed to stressors. The range is one to three. In Smith’s book, there is a table that rates the hardiness of different organisms. *Salmonella, E. coli* and *E. coli* O157:H7, as well as *Campylobacter* spp, *Yersinia* spp., and *Listeria* spp. all are a hardiness rating of one. *Leptospira* spp. and *Streptococcus* (enterococci) are a two and *Clostridium perfringens* and *Mycobacterium* are a three. Data on numbers of pathogens in each category were deemed necessary as an indicator that all pathogens of concern in road-killed deer could be

destroyed during composting. From hardiness level one, fecal coliforms, *E. coli* and *Salmonella* were chosen, and fecal strep and enterococci from level two. *Mycobacterium avium* subspecies *Paratuberculosis* (MAP) was chosen as the level three bacteria.

Clostridium perfringens is not only found in the intestinal tracts of animals, but is also found as part of the micro flora of soil (Smith, 1975). As they are ubiquitous, they are likely to be everywhere. Woods End Research Lab has done *Clostridium* testing and has found it declines rapidly in composting (personal communication). It also sporulates at high temperatures and when exposed to air. It was thus deemed not appropriate for use as an indicator.

Of the three mycobacterium used to set pasteurization standards, *M. bovis* is the most sensitive to heat, followed by *M. avium* and then *M. paratuberculosis* (Sung and Collins, 1998). Depending on the species, mycobacterium may grow at a wide temperature range from 10 to 65^{o}C, though the optimum growth range for most species is between 28 and 45^{o}C (Kusnetsov, et al 2003).

Seeding with lab-reared organisms was the original intention of this study; however, as the literature review progressed and resulted in the selection of study organisms different from those in the original proposal, the methods also evolved. It is doubtful if the behavior of seeded bacteria, viruses and parasites mimics that of naturally occurring organisms (Strauch, 1987). Therefore, since the level one and level two organisms can all be found in the intestinal tract of deer, it was decided to use actual intestinal contents of the road-killed deer for seeding into recoverable bags placed in the compost piles. These organisms will also be studied in the compost itself. Due to the fact that we have a relationship with Veterinary School faculty in the Johne's laboratory, we also have access to manure testing positive for *Mycobacterium avium* subspecies *Paratuberculosis* (MAP). Therefore, this was chosen as the level three bacteria with which to seed carcasses. MAP testing will not be done on the compost though, as it is not found in white-tailed deer. It is being used as an indicator organism.

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