

## Methods and microbial risks associated with composting of animal carcasses in the United States

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Animal carcass composting for both routine and emergency management of dead production animals is an alternative method of carcass disposal in those situations in which conventional methods are inadequate. Carcass composting differs from composting other materials such as manure and green waste and presents some unique challenges. Carcasses are typically composted whole and do not present uniformly chopped substrate for microbial action, and these compost piles are not turned frequently. Both of these factors contribute to a nonuniform compost composition at the end of the process. Although allowances for this nonuniformity need to be made, well-designed carcass compost systems (with proper maintenance and monitoring) do result in a safe and efficient method of disposing of dead animals with minimal environmental impacts. Importantly, proper composting eliminates many pathogens and may reduce levels of carcass contamination with spore-forming bacteria, prions, and other specific pathogens. When considering options, carcass composting should be evaluated via a risk assessment approach that includes all stages of disposal of dead animals, such as handling, transportation, processing, storage, and disposal; among the various disposal systems under consideration, risk comparisons need to account for the sum of risks from the time of death to sequestration or destruction of potential microbial threats associated with an animal carcass.

### The Current Situation in the United States

Part of the challenge associated with the disposal of animal carcasses includes protection of environmental, animal, and public health against potential microbiological threats. An animal carcass is composed of microbiologically active material that may contain viruses, bacteria, protozoa, parasites, prions, toxins, drug resi-

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### ABBREVIATIONS

BSE	Bovine spongiform encephalopathy
log <sub>10</sub>	Logarithm base 10
MRA	Microbial risk assessment
Stx	Shiga toxin

dues, and other chemicals. All of the biologically active materials need to be reduced to safe amounts, eliminated, or sequestered to minimize their potential hazard. Regulations to provide uniform standards for biosecurity, traceability, and environmental protection are necessary. Biosecurity agencies in Australia, New Zealand, the United States, and Canada have recognized the potential benefits of composting for both routine and emergency management of deaths among production animals and have identified it as the preferred method of carcass disposal in certain situations.<sup>1</sup>

The disposal of dead animals is not federally regulated in the United States and varies between and within states. The principal methods of carcass disposal are rendering, burial, incineration, and composting. Lactic acid fermentation, alkaline hydrolysis, and anaerobic digestion are additional options that currently offer limited capacity for disposal.<sup>2</sup> New technologies continue to enter the market, including microwave sterilization and gasification. All methods have strengths and weaknesses.<sup>2</sup> Several federal and state agencies in the United States have regulations pertaining or relevant to the disposal of animal carcasses. More coordination and harmonization of rules among these regulatory authorities would help eliminate confusing and conflicting information. Although composting as a form of routine or emergency animal carcass disposal has been approved in several states, other states have no rules and some prohibit the practice.<sup>3</sup>

Carcass composting has been referred to as “above-ground burial in a bio-filter with pathogen kill by high temperature”.<sup>4</sup> Historically, it is known to be a safe method of disposal of animal manure.<sup>3,5</sup> Compared with carcass composting, the methods for animal manure composting<sup>6-8</sup> and associated risks of disease transmission have been more extensively investigated.

Unusual sudden increases in death rates or other catastrophic losses can exceed the rendering capacity in a local region.<sup>2,9</sup> Such spikes in mortality rates and catastrophic losses may be attributable to epidemic dis-

ease, severe weather, electric and transportation failure, or other emergency situations including quarantine and market interruption. Even temporary interruptions may have high impacts on intensive animal production operations because those operations have limited storage capacity and production time lines that require regular feed delivery and the entry of replacements and exit of market-ready stock. Effective and safe on-farm disposal of dead animals decreases the potential for environmental contamination and disease spread from the biological hazards associated with animal carcasses.

The US rendering industry collects and disposes of most dead animals and unwanted animal by-products. Economic impacts associated with what is known as the feed rule<sup>10</sup> to protect the United States from the threat of BSE have increased the costs of rendering and resulted in a fee for this service to the producer. Consequently, the amount of animal by-products and carcasses that are disposed of on farms without proper safeguards may have increased since 2000. The approved alternative methods of carcass disposal, including pit burial, individual burial, commercial certified landfills, alkaline digestion, carcass composting, and incineration, vary among states.<sup>2,3</sup> Many of the more traditional methods require specialized equipment and appropriate geologic, hydrologic, and climatic conditions. States that do not allow carcass composting limit their options and may lack the capacity for safe and effective carcass disposal when faced with sudden increases in mortality rates or catastrophic losses among production animals.

The interest in the use of on-farm composting for the disposal of animal by-products and carcasses is growing because the practice is relatively simple, effective, environmentally sound, and economic. It uses materials and equipment that are often available or readily accessible on farms. The finished compost can be applied to the land, if permitted by state and local regulatory agencies, thereby providing an environmentally acceptable means of recycling nutrients and stabilized organic matter into the soil. Proper composting of carcasses requires the same expertise to manage the process and site as that required for proper composting of manure, landscape trimmings, and other materials. Improper composting can result in slower digestion of tissues along with adverse environmental impacts, including odor and leachate production as well as inadequate pathogen reduction. Proper compost system design, maintenance, and monitoring are straightforward. In many states, the poultry industry has successfully used composting for carcass disposal under a variety of environmental conditions for nearly 2 decades.

Several states have guidelines and information on the World Wide Web about composting of animal carcasses. Permits for carcass composting are issued on the basis of the type and extent of composting in some states. Certification for composting is granted in certain states on the basis of livestock producer participation in courses. The National Resource, Agricultural, and Engineering Service has excellent guidance publications on composting, and the National Sustainable Agricultural Information services also provide relevant explanatory documents.<sup>11,12</sup> Composting research has been performed at The Ohio State University,<sup>3,13</sup> Texas

Cooperative Extension,<sup>14,15</sup> and the National Agricultural Biosecurity Center at Kansas State University<sup>2</sup> and by the Alberta Provincial government.<sup>11</sup> These groups have provided reviews and databases of resources that describe the economic, environmental, and technical aspects of on-farm composting, including the composting of carcasses. In Canada, a highly biosecure, enclosed system of composting was used by the Canadian Food Inspection Service to deal with thousands of carcasses during an avian influenza outbreak in 2004.<sup>16</sup> The Cornell Waste Management Institute has published an extensive literature review<sup>17</sup> on the expected prevalence and persistence of pathogens in composted New York State roadkill.

## **Principles and Elements of Composting**

Composting is a largely aerobic process in which bacteria, fungi, and other microorganisms convert organic material into stable humus. Composting of animal carcasses requires precautions to minimize the potential spread of diseases, odors, and liquids. The composting process depends on naturally present microorganisms to digest the organic components in the carcass. The carbon-based materials in the composting piles supply energy for the microbes, and the carcass tissues and fluids supply nitrogenous materials for microbial protein synthesis. Heat, water, carbon dioxide, ammonia, and volatile organic compounds are by-products produced in the composting process. Much of the digestion within and at the outer surface of the carcass is anaerobic, but the liquid and gaseous by-products of the anaerobic process diffuse away from the carcass and into progressively more aerobic layers of the composting envelope, where aerobic degradation further reduces them to carbon dioxide and water.

The microbial flora responsible for the decomposition of organic matter are a complex mix of organisms, some of which are able to function and survive at temperatures that are sufficiently high to kill mammalian and avian pathogens.<sup>18-21</sup> These complex microbial decomposer communities occur naturally in the environment, and many of the mesophilic microbes (those that grow best at 20° to 55°C [68° to 131°F]) are responsible for the continuous and normal decay of plant and animal tissues at ambient temperatures. The thermophilic microbes (ie, those that withstand and grow at temperatures > 45°C [113°F]) inhabit naturally self-heating environments such as animal nests, hot springs, and large piles of storm debris. There is no need to add special microbes to the composting matrix.

Composting of all types of organic materials is affected by physical and chemical factors such as the carbon-to-nitrogen ratio, moisture content, oxygen concentration, temperature, and pH.<sup>1,14,22</sup> For optimum carcass composting, recommended conditions include an initial carbon-to-nitrogen ratio in the range of 25:1 to 30:1, an initial moisture content of 50% to 60%, and an initial oxygen concentration > 10%. These conditions facilitate thermophilic composting and supporting temperatures of 43° to 66°C (109.4° to 150.8°F), which are optimal for compost microorganisms.<sup>8,23</sup> Temperatures > 70°C (158°F) or < 40°C (104°F) reduce the thermophilic mi-

crobial population and their accompanying enzymatic activities that are responsible for rapid decomposition and stabilization of the organic feed stocks. At temperatures of 55°C for 3 consecutive days, most pathogenic bacteria and parasites are killed and most viruses are inactivated. Lower temperatures will support carcass decomposition but prolong the duration of the process: as a rule of thumb, general chemical and biochemical reaction rates approximately double with each 10°C (18°F) increase in temperature.<sup>24</sup> The target pH for composting is neutral, although successful composting occurs at pH values of 5.5 to 9.0.<sup>25</sup>

The design of carcass composting systems must address 4 major safety and acceptance issues: protection of ground and surface water, minimization of the risk of spreading disease, prevention of nuisances from scavengers and insects, and maintenance of air quality.<sup>4,15</sup>

Composting systems are divided into open and closed systems.<sup>8</sup> Open systems include windrows, static piles, and bins. The simplest system involves the use of windrows, which have a defined width and variable length as needed. Animal carcasses are added incrementally onto a thick bedding of compostable, absorbent carbonaceous material. As needed, the windrow can be lengthened to accommodate additional large (> 227-kg [500-lb]) carcasses, which are typically composted in a single layer (Figure 1). If carcasses are small, windrow height can be increased with additional layers of carcasses separated by sufficient carbonaceous material to absorb and retain liquids. Composting bins are constructed with 3 permanent walls and are sized to accommodate the equipment used to handle the material (eg, a skid-loader). Material is transferred from the primary phase bin to a secondary phase bin at 10 to 21 days (for poultry) or when soft tissue decomposition is

complete, pile temperature cools, and fresh aeration is needed to continue the self-heating process. Windrow and bin systems are the most common methods used for on-farm carcass composting.

Closed, in-vessel systems are far less common and typically are used for small species (eg, poultry and nursery pigs). In closed systems, the composting mass is contained within an insulated structure; aeration is provided through a series of vents or during rotation if a horizontal drum configuration is used. Technologically advanced composting systems use reactor vessels with mechanical aeration and mixing of material. Composting processes and facilities range from highly mechanized and intensively managed (frequently turned or mechanically aerated) systems used in production industries to attain maximum throughput and minimum processing time to much simpler naturally ventilated static pile systems that decompose materials more slowly and that require much lower capital and operating costs. In some situations, the process may be started in a closed system (in-vessel system) and eventually transferred to an open system to complete the process.

Most carcass-composting operations employ naturally ventilated, static pile processes. In rainy climates, static pile bins are commonly approximately 5 to 6 feet in height and are covered by a roof to prevent water saturation that can interfere with the process. In dry climates, carcass composting is typically done in unsheltered windrows and may require the addition of moisture to maintain optimal digestion. Open windrows are used during emergencies to enhance biosecurity and reduce environmental pollution risks associated with burial of high numbers of animals deaths. During periods with considerable rainfall, additional precautions are required to minimize leachate contamination of the environment from open windrows. These precautions include use of extra thick layers of absorptive material over and beneath the carcasses or covering the windrows with water-shedding fabrics or plastic sheeting.

Animal carcass composting piles are typically constructed in layers, starting with a thick absorptive layer of carbonaceous plant material. Whole carcasses are laid on top of the base and covered with additional absorptive organic material. Succeeding layers of carcasses are added on a daily basis until the bin is filled or until an appropriate freestanding pile height is reached. Bins containing poultry or similar small carcasses may contain many layers. Composting piles or bins may include 2 or 3 layers of mature sheep and swine carcasses, whereas mature cattle are usually composted in a single layer with 2 carcasses placed back to back (Figure 1). The practice of opening the carcasses of ruminants by lancing the rumen and thorax has been questioned. Although

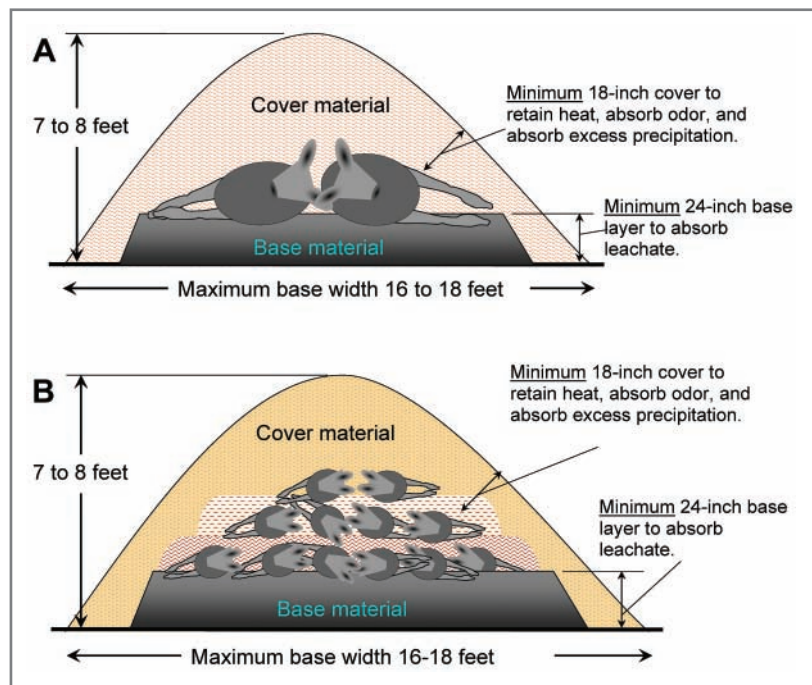


Figure 1—Illustration of the placement of large carcasses (cattle and horses; A) and small carcasses (swine, sheep, calves, or poultry; B) and in a static pile composting system.

lancing may prevent the carcass from exploding, which may increase the risk of disease spread, the procedure of lancing could potentially also increase the risk of disease spread. It has been suggested that such lancing does not enhance the overall composting process or outcome.<sup>1</sup> A layer of absorptive carbon material that is approximately the same thickness as the carcasses is used between each carcass layer to retain the heat produced by microbial activity and to absorb excess liquid released from the carcasses during digestion.

The success of naturally ventilated static pile composting processes depends on the characteristics and thickness of the materials used to envelope the carcasses. In reports<sup>26,27</sup> of a 3-year study of emergency cattle carcass-composting procedures, it was concluded that water-holding capacity, biodegradability (for heat production), gas permeability (for oxygen penetration), and mechanical strength (to prevent compaction and loss of gas permeability) are the most important envelope-material factors. Particle size can be used as a practical proxy measure for the all-important gas permeability that permits air to flow into compost. Particle sizes of envelope materials in the 0.6- to 5-cm (0.25- to 2.0-inch) range have been found to work reasonably well on the basis of field observations. Smaller particle sizes restrict gas movement, and larger sizes can lead to excessive airflow and chilling of the composting process during cool weather. Experience indicates that many wood by-products and crop residues are effective in poultry and livestock composting. Some common materials include sawdust, wood chips, ground cornstalks, rice hulls, ground straw, corn silage, straw-ma-nure mixtures, and poultry litter.

Composting times vary depending on the size of the carcasses, ambient temperature, and other physiologic factors. Undisturbed primary composting refers to the first peak-heating phase inclusive of the gradual cooling that ensues. The duration of the primary phase will vary depending on carcass size. The estimated duration of primary composting ranges from 10 days for fowl to 195 days for adult bovinds.<sup>4</sup> Primary composting is recommended for all carcasses to minimize the spread of infection and allow for breakdown of soft tissue. Following the primary composting period and cooling to 45° to 48°C (113.0° to 118.4°F), the compost can be turned to stimulate the secondary compost heating phase in which bones will be degraded. Secondary composting is performed for an additional period of 10 to 65 days. The design of an effective composting structure or windrow has been previously described<sup>28</sup> and is applicable for any animal that weighs 2 to 650 kg (4.4 to 1,430 lb). The period required for animal decomposition can be roughly estimated through a function of carcass weight; the required time interval can be calculated by use of the Keener equation as follows:  $T = 7.42 \times W^{0.5} \geq 10$ , where T is the primary composting time (in days), and W is the weight of the heaviest carcass. This formula is based on a variety of carcass composting demonstration projects, many of which were carried out in warm climates or during warm seasons. However, external temperatures play a highly important role in decomposition time and are not reflected by the Keener equation.

Turning the compost mass infuses it with a supply of oxygen, thereby supporting thermophilic digestion. However, turning piles with large animal carcasses is difficult and unappealing. Often, carcass compost masses are not turned; if the pile is turned, this typically occurs only after the primary composting cycle is complete. As a result, these piles retain a layered structure, which results in considerably higher spatial variation within the pile. Oxygen concentration, for example, which is supplied by natural diffusion from the outer surface, is maximal in the exterior part of the envelope of carbon-based material and low within the core, where areas of dense tissue are located. However, temperatures are often highest near the core because of the extensive insulating effect exerted by the envelope material and lower near the walls or edges of the pile, particularly during cool weather. The carbon-to-nitrogen ratio is usually low at the core near the carcasses because of the high nitrogen content of animal carcasses, and the ratio gradually increases toward the periphery of the compost pile. Similarly, moisture levels are usually greatest in the immediate vicinity of fresh carcasses and at the base of the pile and decline gradually to relatively dry conditions in the outer envelope as a result of the wicking action of the surrounding carbon-based envelope material.

Simple design and construction guidelines greatly improve the potential to achieve important biosecurity and environmental goals. In the final analysis, these goals can be met by sheltering the piles from excess precipitation and high wind and by application of sufficient quantities of acceptable envelope material. Achieving and maintaining temperatures that reduce pathogen survival depend mainly on use of a sufficient thickness of outer envelope materials that have good insulating properties. Similarly, leachate retention depends on use of a sufficient thickness of materials that have high water-holding capacity in the base of the pile. Although oxygen concentrations in the core of unturned piles are likely to be less than the 5% minimum, use of envelope materials cut to a particle size to provide sufficient free airspace and gas permeability can ensure that the outer envelope of the pile sustains an aerobic microbial bio-filter layer that decomposes odorous compounds before they are released into the atmosphere.

Livestock carcass-composting facilities should be sited so as to not cause pollution of surface water, groundwater, or soil. Provisions are needed for containment or appropriate diversion and collection of leachate and surface runoff from the pile and, if necessary, subsequent appropriate treatment. The site should be at least 100 m from any water source on public or other private property, but not in a low-lying tract of land. A lime-stabilized clay,<sup>29</sup> asphalt, or concrete surface is recommended to facilitate year-round access and prevent pollution of soil beneath the compost piles.

Regular monitoring of the compost operation is essential. This includes ensuring that all parts of a carcass are properly covered at all times, which often necessitates the addition of envelope material should shift or collapse of the compost pile occur. Temperature monitoring, preferably assessed at the carcass surface, is a key indicator of a properly functioning compost pile,

and daily monitoring is recommended to ensure that the temperature increases to the optimal value; thereafter, at least weekly monitoring should be performed to ensure stable composting conditions. Records of the composting piles temperatures should be maintained. Temperatures should increase from 55° to 66°C and remain in this range for at least 1 week. The time necessary to achieve optimal temperature is dependent on external ambient temperature and on the heat-producing and heat-losing characteristics of the co-compost. Reductions in compost pile temperature from 45° to 48°C indicate a need for more oxygen or substrate. Turning a pile too early can release odor, chill the pile, and increase the risk of pathogen release. Compared with composting procedures for small carcasses, a longer interval should be allowed to elapse before turning a pile containing large carcasses is considered. The frequent turning and aeration practices used in municipal and industrial composting facilities are less important in smaller composting operations with lower carcass-processing loads; compost pile turning may actually disturb and slow down the process if done too frequently or at the wrong time. Turning, in fact, is not necessary at all if the envelope material has good mechanical strength and gas permeability.<sup>26</sup> Odors may indicate a failed composting process. Excessive loss of leachate from the compost may indicate excessive moisture in the pile and insufficient use of absorptive cover beneath and between the carcasses.

### **Microbial Risks Associated with Composting of Animal Carcasses**

A wide variety of potential microbial pathogens are found in manure, food waste, and animal carcasses. Microbial die-off and survival associated with composting animal carcasses and other organic wastes at locations throughout the world have been investigated. Bacterial pathogens, unlike viruses and parasites, can survive outside the host organism if composting temperatures are inadequate for their destruction. An additional concern is the potential for regrowth of organisms that were not completely eliminated if conditions subsequently become favorable. Ova of the parasite *Ascaris lumbricoides* are especially resistant to destruction and have therefore been accepted as a benchmark or proxy for microbial destruction achieved by various treatment systems. Bacterial pathogens potentially found in meat, food scraps, manure, sludge, and other organic residuals will be destroyed by exposure to the time-temperature regimens attained in a well-managed composting environment (Appendix 1). The static compost pile coupled with the nonuniform composition of carcass compost presents special conditions that warrant additional research into the potential risks of spore-forming bacteria, materials handling, and the final disposition of the compost product. Laboratory-scale experiments have indicated that enteric pathogens such as *Salmonella* spp and *Escherichia coli* O157:H7 in bovine fecal matter are inactivated at thermophilic composting temperatures.<sup>36-38</sup> Similarly, windrow composting of spent broiler litter resulted in at least 6 log<sub>10</sub> reductions in numbers of total coliforms, fecal coliforms, *E coli*, and

fecal *Enterococcus* spp.<sup>39</sup> Another study<sup>40</sup> of composting of municipal solid waste has revealed similar trends.

Survival of Stx-encoded bacteriophage in a compost model was measured to evaluate public health risks of compost use as a soil amendment on land used to grow food crops.<sup>41</sup> Results of that study<sup>41</sup> indicated that Stx-encoding phages are quickly eliminated (undetectable after 3 days) in cow manure compost in which peak temperature was 60°C (140°F). In contrast, another study<sup>42</sup> revealed that Stx-encoding phages in the environment were more environmentally persistent and resistant to chlorination and heat treatment than their host *E coli*.

Regrowth of salmonellae and coliform bacteria in mesophilic conditions<sup>43,44</sup> in products that are incompletely stabilized (ie, those containing labile, easily biodegradable, and nonhumified organic fractions<sup>45</sup>) has been reported. In contrast, results of several studies have indicated that *Salmonella* regrowth is strongly suppressed by competing microflora in compost soil<sup>35,46-48</sup> and sludge.<sup>49</sup> However, when compost was tested after being stored for longer than 2 years, the rate of *Salmonella* inactivation was reduced, compared with that observed in composts after 2 to 33 weeks.<sup>50,51</sup> By 117 weeks, the maximum amount of *Salmonella* growth was quite low (90 to 175 most probable number/g), in contrast to the regrowth counts in compost at 2 to 65 weeks (1,400 to 9,800 most probable number/g). Examination of uncovered storage of municipal solid waste windrow compost<sup>52</sup> and dried sewage sludge compost<sup>53</sup> confirmed recovery of certain enteric bacteria and salmonellae. In the latter study, serotyping of the *Salmonella* organisms in the stored, dried biosolids (detected only in samples collected after many weeks) revealed that they were distinct from those in the original biosolids; thus, the conclusion was that wildlife was the source of the pathogen. By use of DNA gene probes to evaluate survival of *Salmonella* ser Typhimurium and *E coli*, differences in pathogen survival between industrial- and laboratory-scale composting operations have been evaluated.<sup>54</sup> In bench-scale trials involving food wastes, both of those species of bacteria survived for 9 days at processing temperatures of 60° to 70°C. However, during industrial trials, both species survived for 59 days at processing temperatures of approximately 60°C. Both species ultimately became undetectable after temperatures were decreased to approximately 40°C during compost curing. The investigators concluded that temperature and time of exposure were difficult to correlate with destruction of pathogens and that the mechanism for removal of these microorganisms during aerobic composting is "complex and not simply the result of a thermal physical environment."<sup>54</sup> The important thing to note from those studies is that the more stable products with low available carbon content and an actively respiring competitive microbial population will decrease the amount of regrowth and hasten the die-off of the regrowth populations. It is also evident that regrowth populations rarely become equivalent to the peak populations present in the untreated materials because of lower concentrations of available nutrients and increased numbers of competitive bacteria.

In a study<sup>55</sup> of rural human sewage sludge composting in France, a straw envelope was placed around the pile and turning was performed monthly; as a result, efficient elimination of nematode eggs, enteroviruses, fecal indicators (*Enterococcus* spp and *E coli*), and pathogenic bacteria (*Salmonella* spp, *Listeria monocytogenes*, and *Clostridium perfringens*) was achieved. Temperatures in the bottoms of the piles were the lowest (< 50°C [122°F]), compared with temperatures in other areas (< 66°C); however, location within the pile had no effect on microorganism survival.<sup>55</sup> The number of *C perfringens* decreased gradually from an initial density of approximately  $8 \times 10^4$  colony-forming units/g of dry solids to approximately  $7.6 \times 10^2$  colony-forming units/g of dry solids (the number of *E coli* similarly changed) after 6 months of composting. Biowaste recycling has been implicated as an animal and public health hazard with regard to pathogenic anaerobic spore formers (eg, *Clostridium botulinum*). In 1 study,<sup>56</sup> samples of marketed biocompost in Germany were tested and results indicated that approximately 50% of the tested samples contained *C botulinum*. Also, findings indicated that household biowaste collection in so-called bio-bins was a risk factor for the production of contaminated compost end-products, but that high composting standards and management could minimize the risk.<sup>56</sup>

Because of its potential for toxic effects in humans and other animals, concerns have been expressed about proliferation of *C botulinum* in anaerobic zones during the initial first few days after a carcass compost pile is constructed prior to onset of thermophilic stages. Important facts about the types of this bacterium, their characteristics, and toxin production provide perspective in evaluation of these concerns.

*Clostridium botulinum* is found worldwide in soils; sediments; intestinal tracts of birds, fish, and mammals; and decaying wildlife carcasses and the insect larvae associated with them as well as on vegetation that contacts contaminated soil.<sup>57-59</sup> This anaerobic bacterium produces an extremely potent proteinaceous neurotoxin, which is released when its heat-resistant endospores germinate. Of the 7 types of toxins (A through G) produced by *C botulinum*, only types A, B, E, and, rarely, F, affect humans, whereas types C, D, and G are toxic to other animals.<sup>59</sup> Some nontoxigenic, proteolytic strains of *C botulinum* (designated *Clostridium sporogenes*) that are present in soil sediments inhibit germination of *C botulinum* spores and destroy the toxin.<sup>60,61</sup> Also, some strains of *C perfringens*, which are present along with *C botulinum* in soil, produce inhibitors that negatively affect germination of spores of *C botulinum* types A and B.<sup>62</sup>

Both *C botulinum* and *C perfringens* are regarded as human pathogens and important food spoilage organisms associated with dairy, meat, and poultry products as well as fresh and canned fruits and vegetables. Spoilage and illness result when products are inadequately heat treated or temperature abused. The source of contamination is thought to be spores in soil residue. *Clostridium botulinum* has been associated with livestock toxicoses following consumption of inadequately fermented (pH > 4.5), contaminated haylage.<sup>63</sup> In addition, wildfowl flock deaths as a result of ingestion of preformed toxin in stagnant water or insect larvae have been reported.<sup>64,65</sup>

In a carcass-composting environment, an anaerobic zone is expected to develop during the early phase of the process, especially with large ruminant carcasses. Until the interior temperature reaches 45° to 47°C (113° to 116.6°F), the anaerobic zone will support vegetative growth of any *C botulinum* that may be present. However, any toxin released from germination of preexisting spores would be inactivated by the ruminal bacterial flora<sup>65</sup> and the developing thermophilic temperatures. As other decomposer bacteria grow throughout the compost pile, the temperature increases and general microbial competition occurs; consequently, *C botulinum* growth slows and the survival response will stimulate sporulation.

Although some spores may germinate and release toxin, others will only partially germinate because of suboptimal conditions, including suboptimal temperatures, inadequate nutrient supply, and presence of inhibitors. Optimal temperatures for germination and toxin release for *C botulinum* types A, C, and E are 38° to 40°C (100.4° to 104.0°F), 40° to 42°C (104.0° to 107.6°F), and 33° to 35°C (91.4° to 95.0°F), respectively.<sup>63</sup> For any toxin released, it will be either immediately or subsequently exposed to thermophilic temperatures because carcass compost piles are constructed so that the animal tissue lies in the core, which heats maximally. Thus, the toxin is inactivated as the temperature and pH increase. Overall, spore germination is followed directly or subsequently with heat inactivation of both toxin and any remaining vegetative cells, which results in breakage of the propagation-sporulation cycle. Rapid spore germination (ie, within 2 hours) at 60°C has been observed for some strains.<sup>66</sup> Because of the lack of suitable conditions for vegetative growth in a thermophilic composting pile, the outcome for continued propagation at these high temperatures with increasingly aerobic conditions as the composting proceeds is problematic. As a result, spore numbers will decline from the peak value that developed during the initial periods. In a recent report<sup>55</sup> on *C perfringens* in sewage sludge compost, viability of anaerobic spore-forming *Clostridium* spp was significantly reduced in thermophilic composting situations.

Some *C botulinum* spores likely survive in micro-pockets of the final composting mass because of low temperature and anaerobic zones; when the pile is turned and reheating occurs, these spores are faced with the aforementioned survival stressors. Any remaining spore count will be reduced during further exposure to heat, aeration, and microbial competition. The lack of remaining animal tissue in the final compost product reduces the attractiveness of the material to wildlife for scavenging. This limits the likelihood of ingestion and spread of the organism by this means. The final product can also be reserved for use as cover or base material for subsequent compost piles, thereby keeping the material within the immediate composting area.

Remaining spores would become part of the soil site upon which the compost is spread; these spores would increase the population of *C botulinum* spores that is already present in the soil. Following the type of scenarios and risk analyses for land application of catering waste used by Gale,<sup>67,68</sup> the risk of substantially

increasing the environmental and soil populations of *C botulinum*, compared with the existing background populations, appears small.

In a simulation study,<sup>69</sup> the effects of 3 manure-handling methods (thermophilic composting at 55°C, manure packing at 25°C [77°F], and liquid lagoon storage) on *Mycobacterium avium* subsp *paratuberculosis*, *Salmonella* spp, *E coli*, and *Listeria monocytogenes* were investigated. *Mycobacterium avium* subsp *paratuberculosis* DNA was detectable through day 56 in manure samples treated by each method, but no bacteria were cultured after day 0. After 3 days of composting, none of the other zoonotic bacteria were recovered via bacterial culture. Composting was associated with a higher level of pathogen inactivation, compared with the other 2 methods, and was therefore recommended for treatment of manures destined for pathogen-sensitive environments such as rapidly draining fields and areas used for vegetable production or residential gardening. The inactivation of *M avium* subsp *paratuberculosis* in composted manure was also evident in a study<sup>70</sup> on 2 farms in New York State.

Most available data indicate that composting efficiently eliminates viral agents. Parvovirus and enterovirus were effectively eliminated after 28 days during composting of cow manure for land application.<sup>71</sup> There have been concerns about prion agents remaining in compost. A study<sup>72</sup> of the effect of composting on prions revealed that there may be degradation of prions during composting, providing evidence of another safety advantage of composting.

An MRA could quantify the probability of a harmful effect of composting in humans, other animals, and the environment. An MRA can be qualitative or quantitative and can identify areas for further research. Furthermore, an MRA can provide estimates of the magnitude and likelihood of an adverse event, such as the spread of disease through composting.

A quantitative MRA was performed in the United Kingdom to evaluate the risks to farm animals from pathogens in composted catering waste that contained meat.<sup>68</sup> The investigation included assessment of BSE, foot-and-mouth disease, African swine fever, and classical swine fever by use of a quantitative MRA developed for assessment of BSE in sewage sludge.<sup>39,73</sup> It was concluded that the important factors governing risk were sources of composted animal parts, the efficiency of composting, and the decay and dilution in soil when compost was spread on pasture. The net pathogen destruction was heavily influenced by the degree of bypass, which is the compost that does not reach critical temperature because of its location in the pile. Even if an assumption of zero reduction of BSE in compost was applied, composting and compost spread on pasture were deemed safe when a 2-tier (primary and secondary) composting system was used together with a 2-month grazing ban for the treated pasture. The study<sup>67</sup> concluded that CSF constituted the highest risk, but that by use of a 2-tier composting system and a 2-month grazing ban, the risk could be as low as 1 pig/very 190 years in the United Kingdom.

In a situation in which a method of carcass disposal is evaluated for possible approval, it is necessary to es-

timate and compare the risks of that method with those associated with alternate methods of carcass disposal that are currently approved, such as rendering and incineration. For example, when evaluating risks of on-farm composting, composting has to be evaluated and compared with the rendering process and also against the transport and handling of carcasses prior to rendering.

The transportation of fallen stock (animals that die or are euthanatized other than at slaughter) from the premises of origin to a site of further processing or disposal may be associated with risks for spread of contagious diseases. In a study<sup>74</sup> to evaluate risk factors for the spread of low pathogenicity H7N2 avian influenza virus among commercial poultry farms in Virginia, it was found that transportation of carcasses for rendering increased the risk of spread of avian influenza, and composting of carcasses was recommended. Recently, a renderer in Australia developed anthrax as a result of handling of a *Bacillus anthracis*-infected carcass.<sup>75</sup> During an outbreak of anthrax in Saskatchewan in 2006, more than two thirds of the bovine carcasses were burnt and the remainder were buried. No carcasses were transported off-site for disposal, and the burial and burning occurred on the same pastureland on which a given animal died.<sup>8</sup> Buried *B anthracis*-contaminated carcasses have been the suspected causative factor in several anthrax outbreaks.<sup>76</sup> This indicates that although carcass composting may not eliminate *B anthracis* spores, alternative methods of carcass disposal also present risks to humans and other animals.

### **Equipment and Methods for Composting Studies**

In many instances, evaluation of composting as a means of carcass disposal has been based simply on recovery of microorganisms (with whatever limits of detection are associated with the recovery method) at various stages during composting. Variable times of sample collection, sampling strategies, and microbiological detection methods make comparisons among studies and evaluation of the safety of composting difficult.

Detection of microbial pathogens has become increasingly efficient, and as such, the criteria for zero risk have become increasingly difficult to meet. Therefore, in assessments of composting systems, a relative risk reduction or a risk limit should be defined. The risk reduction estimates obtained from the risk assessment should be compared with standards such as those recommended by the European Food Safety Agency,<sup>77</sup> and acceptable endpoints for microbial burden reduction need to be determined. The European Food Safety Agency Biohazard Panel recommended that a process can be approved if it meets 3 criteria: 5 log<sub>10</sub> reductions in the number of non-spore-forming pathogenic bacteria, parasites, and nonthermoresistant viruses; 3 log<sub>10</sub> reductions in infectivity titer of thermo-resistant viruses; and 3 log<sub>10</sub> reductions in the number of parasites (viable stages).

In microbiological studies<sup>78,79</sup> of compost toilets, in situ measurements and indicator bacteria were evaluated in sentinel chambers. In a study<sup>80</sup> of sanitation of human feces, thermophilic composting and ammonia-based treatment were evaluated and compared with storage treatment. Thermal composting of fecal matter and food waste in a 90-L reactor resulted in a treatment

temperature > 65°C (149°F). By use of insulation and turning the compost 3 times during the high-temperature period, it was possible to ensure 5 log<sub>10</sub> reductions in numbers of pathogens. A new method to mathematically evaluate and estimate the safety margins of pathogen inactivation during thermal composting has been developed.<sup>80</sup> A laboratory-scale composting reactor has been constructed for systematic studies of the effects of oxygen concentrations and temperature on carbon and nitrogen turnover in household waste compost; this reactor is equipped for independent control of oxygen concentration and temperature.<sup>81</sup> On the basis of the accumulated data, it appears that composting may be inhibited by an excessively rapid increase in temperature, and an improvement of the composting time for household waste during an initial low-pH phase by mesophilic temperature control has been achieved.<sup>82</sup>

Another study<sup>18</sup> investigated the use of a forced-aerated in-vessel system (55-L volume) to compost food waste, cow manure, and bulking materials (wood shavings and mulch hay). A statistical extreme vertices mixture design method was used to design the composting experiments and analyze the collected data.<sup>18</sup> Maximum temperature values of the mixtures were used as a response for both extreme vertices mixture design and statistical analyses. Chemical changes (moisture content, carbon-to-nitrogen ratio, concentration of volatile solids, and pH) and reductions of indicator (fecal coliforms and fecal streptococci) and pathogenic (*Salmonella* spp and *E coli* O157:H7) microorganisms were measured by use of the most probable-number method before and after a 12-day composting period.

Two methods were evaluated for the sanitary process of full-scale industrial composting: spot testing, in which samples are collected directly from the raw material and then periodically throughout the process for evaluation of the numbers of fecal coliforms, *E coli*, *Enterococcus* spp, and *Salmonella* spp, and direct process evaluation, in which specific organisms (*E coli* and *Enterococcus faecalis*) were inoculated in the raw material and thereafter monitored throughout the process.<sup>83</sup> The direct process evaluation was shown to be a more valuable tool for identifying factors for process optimization in different zones and detecting pathogens that are not typically present in raw material. However, the process is not reliable for evaluation of the overall sanitary process because it is difficult to represent a heterogeneous environment when inoculating a limited number of decomposition zones. The use of indirect process variables (dry matter content, organic matter content, pH, and carbon-to-nitrogen ratio) were found to be unreliable indicators of the sanitation process.

Airborne bacterial risks associated with animal waste handling have been assessed during land application of sewage sludge by use of glass impingers.<sup>84,b</sup> Although airborne salmonellae, fecal coliforms, or coliphages were not detected, data indicated that there were risks for pathogenic *Clostridia* spp and H<sub>2</sub>S-producing organisms in locations undergoing high levels of physical agitation. It appears that *Clostridia* spp and H<sub>2</sub>S-producing organisms are better indicators of airborne sewage or sludge-derived material than traditionally employed bacterial indicators such as fecal coliforms or *Streptococcus* spp.

## Overview

Carcass composting, when done correctly with proper attention to the design, layout, monitoring, maintenance, and environmental impacts of the system used, may be considered an efficient and safe method of disposing of animal carcasses. Composting achieves adequate levels of microbial pathogen reduction, although spore-forming bacteria and prion agents may not be completely eliminated. Further studies are encouraged to determine the effects of composting on spore-forming bacteria and prion agents in carcasses. Federal and state agencies are encouraged to evaluate carcass composting via a risk assessment approach that involves consideration of all stages of the process (including transportation, treatment, and storage of animal carcasses and compost) and to compare the risks associated with composting with those associated with other methods of carcass disposal.

- a. Stephens S, Canadian Food Inspection Agency, Saskatoon, SK, Canada: Personal communication, 2007.
- b. All-glass impinger (AGI-30), Ace Glass, Vineland, NJ.

## References

1. Wilkinson KG. The biosecurity of on-farm mortality composting. *J Appl Microbiol* 2007;102:609–618.
2. Carcass disposal: a comprehensive review. Available at: [fss.k-state.edu/FeaturedContent/CarcassDisposal/CarcassDisposal.htm](http://fss.k-state.edu/FeaturedContent/CarcassDisposal/CarcassDisposal.htm). Accessed Mar 17, 2008.
3. Sander JE, Warbington MC, Myers LM. Selected methods of animal carcass disposal. *J Am Vet Med Assoc* 2002;220:1003–1005.
4. Keener HM, Foster SS, Moeller SJ. Ohio's farmstead composting program—a decade of success. Available at: [www.oardc.ohio-state.edu/ocamm/Keener-Maine%20Mortality%20Paper%205-24-05.pdf](http://www.oardc.ohio-state.edu/ocamm/Keener-Maine%20Mortality%20Paper%205-24-05.pdf). Accessed Mar 17, 2008.
5. Jones PW. Health hazards associated with the handling of animal wastes. *Vet Rec* 1980;106:4–7.
6. *Integrated animal waste management*. Task Force report No. 128. Ames, Iowa: Council for Agricultural Science and Technology; 1996.
7. *Composting for manure management*. BioCycle report. Emmaus, Pa: JG Press Inc, 1998.
8. Chapter 4: composting methods. In: Rynk R, ed. *On-farm composting handbook*. Ithaca, NY: Northeast Regional Agricultural Engineering Service, 1992;24–40.
9. FOXNews.com. California heat wave causes livestock carcass pileup. Available at: [www.foxnews.com/story/02933205661,00.html](http://www.foxnews.com/story/02933205661,00.html). Accessed Mar 17, 2008.
10. Title 21 *Code of Federal Regulations* 589.2000. Available at [www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=589.2000](http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=589.2000). Accessed Sep 3, 2008.
11. On-farm composting: a review of the literature. Available at: [www1.agric.gov.ab.ca/\\$department/deptdocs.nsf/all/sag2147?opendocument](http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/sag2147?opendocument). Accessed Mar 17, 2008.
12. Farm-Scale composting resource list. Available at: [attra.ncat.org/attra-pub/farmcompost.html](http://attra.ncat.org/attra-pub/farmcompost.html). Accessed Mar 17, 2008.
13. Compost Research Group. OARDC FABE. Available at: [www.oardc.ohio-state.edu/michel/CompostResearch.htm](http://www.oardc.ohio-state.edu/michel/CompostResearch.htm). Accessed Mar 17, 2008.
14. Kalbasi A, Mukhtar S, Hawkins SE, et al. Carcass composting for management of farm mortalities: a review. *Compost Sci Util* 2005;13:180–193.
15. Kalbasi A, Mukhtar S, Hawkins SE, et al. Design, utilization, biosecurity, environmental and economic considerations of carcass composting. *Compost Sci Util* 2006;14:90–102.
16. Spencer JL, Rennie B, Guan J. Emphasis on biosecurity for composting poultry and manure during an outbreak of highly pathogenic avian influenza in British Columbia. *Can Anim Health Net Bull* 2004;9:21–23.

17. Prevalence of persistence of pathogens in New York State road-kill disposed of through composting: a literature review. Available at: [cwmi.css.cornell.edu/](http://cwmi.css.cornell.edu/). Accessed Mar 17, 2008.
18. Cekmecelioglu D, Demirci A, Graves RE. Feedstock optimization of in-vessel food waste composting systems for inactivation of pathogenic microorganisms. *J Food Prot* 2005;68:589–596.
19. Nakasaki K, Sasaki M, Shoda M, et al. Effect of seeding during thermophilic composting of sewage sludge. *Appl Environ Microbiol* 1985;49:724–726.
20. Nakasaki K, Sasaki M, Shoda M, et al. Characteristics of mesophilic bacteria isolated during thermophilic composting of sewage sludge. *Appl Environ Microbiol* 1985;49:42–45.
21. Nakasaki K, Nag K, Karita S. Microbial succession associated with organic matter decomposition during thermophilic composting of organic waste. *Waste Manag Res* 2005;23:48–56.
22. Glanville TD, Trampel DW. Composting alternative for animal carcass disposal. *J Am Vet Med Assoc* 1997;210:1116–1120.
23. Nakasaki K, Shoda M, Kubota H. Effect of temperature on composting of sewage sludge. *Appl Environ Microbiol* 1985;50:1526–1530.
24. Haug RT. *Practical handbook of compost engineering*. Ann Arbor, Mich: Lewis Publishers, 1993.
25. Nakasaki K, Yagushi H, Sasaki Y, et al. Effects of pH control on composting of garbage. *Waste Manag Res* 1993;11:117–125.
26. Glanville TD, Richard TL, Harmon JD, et al. *Environmental impact and biosecurity of composting for emergency disposal of livestock mortalities*. Final project report for Iowa Department of Natural Resources. Ames, Iowa: Iowa State University, 2006.
27. Ahn HK, Richard TL, Glanville TD, et al. Laboratory determination of compost physical parameters for modeling airflow characteristics. *Waste Manag* 2008;28:660–670.
28. Keener HM, Elwell DL, Monnin MJ. Procedures and equations for sizing of structures and windrows for composting animal mortalities. *Appl Eng Agric* 2000;16:681–692.
29. Sikora LJ, Francis H. Building a pad from lime-stabilized soil. *Biocycle* 2000;41:45–47.
30. Sorqvist S. Heat resistance in liquids of *Enterococcus* spp., *Listeria* spp., *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella* spp. and *Campylobacter* spp. *Acta Vet Scand* 2003;44:1–19.
31. Byrne B, Dunne G, Bolton DJ. Thermal inactivation of *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spores in pork luncheon roll. *Food Microbiol* 2006;23:803–808.
32. International Commission of Microbiological Specifications for Foods (ICMSF). Microbiological specifications of food pathogens. In: *Microorganisms in foods 5: characteristics of microbial pathogens*. London: Blackie Academic & Professional, 1996:66–111.
33. Rose JB, Sličko TR. *Giardia*, *Cryptosporidium*, and *Cyclospora* and their impact on foods: a review. *J Food Prot* 1999;62:1059–1070.
34. Day M, Shaw K. Biological, chemical and physical processes of composting. In: Stofella P, Kahn B, eds. *Compost utilization in horticultural cropping systems*. Boca Raton, Fla: Lewis Publishers, 2000;17–50.
35. Burge WD, Enkiri NK, Hussong D. *Salmonella* regrowth in compost as influenced by substrate. *Microb Ecol* 1987;14:243–253.
36. Jiang X, Morgan J, Doyle MP. Thermal inactivation of *Escherichia coli* O157:H7 in cow manure compost. *J Food Prot* 2003;66:1771–1777.
37. Jiang X, Morgan J, Doyle MP. Fate of *Escherichia coli* O157:H7 during composting of bovine manure in a laboratory-scale bioreactor. *J Food Prot* 2003;66:25–30.
38. Lung AJ, Lin CM, Kim JM, et al. Destruction of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in cow manure composting. *J Food Prot* 2001;64:1309–1314.
39. Mohee R, Driver MF, Sobrater N. Transformation of spent broiler litter from exogenous matter to compost in a sub-tropical context. *Bioresour Technol* 2008;99:128–136.
40. Hassen A, Belguith K, Jedidi N, et al. Microbial characterization during composting of municipal solid waste. *Bioresour Technol* 2001;80:217–225.
41. Johannessen GS, James CE, Allison HE, et al. Survival of a Shiga toxin-encoding bacteriophage in a compost model. *FEMS Microbiol Lett* 2005;245:369–375.
42. Muniesa M, Lucena F, Jofre J. Comparative survival of free shiga toxin 2-encoding phages and *Escherichia coli* strains outside the gut. *Appl Environ Microbiol* 1999;65:5615–5618.
43. Russ CF, Yanko WA. Factors affecting salmonellae repopulation in composted sludges. *Appl Environ Microbiol* 1981;41:597–602.
44. Mote CR, Emerton BL, Allison JS, et al. Survival of coliform bacteria in static compost piles of dairy waste solids intended for freestall bedding. *J Dairy Sci* 1988;71:1676–1681.
45. Thompson WH, Legee PB, Millner PD, et al. Test methods for the examination of composting and compost (TMECC) on CD, June 2002. Holbrook, NY: *The Composting Council Research and Education Foundation*. Available at: [tmecc.org/](http://tmecc.org/). Accessed Mar 17, 2008.
46. Hussong D, Burge WD, Enkiri NK. Occurrence, growth, and suppression of salmonellae in composted sewage sludge. *Appl Environ Microbiol* 1985;50:887–893.
47. Lang N, Smith S, Bellett-Travers DM, et al. Decay of *Escherichia coli* in soil following the application of biosolids to agricultural land. *Water Environ Manage J* 2003;17:23–28.
48. Millner PD, Powers KE, Enkiri NK, et al. Microbially mediated growth and suppression and death of *Salmonella* in composted sewage sludge. *Microb Ecol* 1987;14:225–265.
49. Zaleski KJ, Josephson KL, Gerba CP, et al. Potential regrowth and recolonization of salmonellae and indicators in biosolids and biosolid-amended soil. *Appl Environ Microbiol* 2005;71:3701–3708.
50. Sidhu J, Gibbs RA, Ho GE, et al. Selection of *Salmonella typhimurium* as an indicator for pathogen regrowth potential in composted biosolids. *Lett Appl Microbiol* 1999;29:303–307.
51. Sidhu J, Gibbs RA, Ho GE, et al. The role of indigenous microorganisms in suppression of *Salmonella* regrowth in composted biosolids. *Water Res* 2001;35:913–920.
52. Déportes I, Benoit-Guyod JL, Zmirou D, et al. Microbial disinfection capacity of municipal solid waste (MSW) composting. *J Appl Microbiol* 1998;85:238–246.
53. Zaleski KJ, Josephson KL, Gerba CP, et al. Potential regrowth and recolonization of salmonellae and indicators in biosolids and biosolid-amended soil. *Appl Environ Microbiol* 2005;71:3701–3708.
54. Droffner ML, Brinton WF. Survival of *E. coli* and *Salmonella* populations in aerobic thermophilic composts as measured with DNA gene probes. *Zentralbl Hyg Umweltmed* 1995;197:387–397.
55. Pourcher AM, Morand P, Picard-Bonnaud F, et al. Decrease of enteric micro-organisms from rural sewage sludge during their composting in straw mixture. *J Appl Microbiol* 2005;99:528–539.
56. Bohnel H, Lube K. *Clostridium botulinum* and bio-compost. A contribution to the analysis of potential health hazards caused by bio-waste recycling. *J Vet Med B Infect Dis Vet Public Health* 2000;47:785–795.
57. Critchley EM. A comparison of human and animal botulism: a review. *J R Soc Med* 1991;84:295–298.
58. Staempfli H, Oliver O. Disease caused by *Clostridium* species. In: Howard J, ed. *Current veterinary therapy 3*. Philadelphia: WB Saunders Co, 1993;568–569.
59. Wells C, Wilkins T. Clostridia: spore-forming anaerobic bacilli. In: Baron M, ed. *Medical microbiology*. 4th ed. Galveston, Tex: The University of Texas Medical Branch, 2008. Available at: [www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mmed.chapter.1050](http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mmed.chapter.1050). Accessed Sep 17, 2008.
60. Graham JM. Inhibition of *Clostridium botulinum* type C by bacteria isolated from mud. *J Appl Bacteriol* 1978;45:205–211.
61. Sandler RJ, Rocke TE, Samuel MD, et al. Seasonal prevalence of *Clostridium botulinum* type C in sediments of a northern California wetland. *J Wildl Dis* 1993;29:533–539.
62. Smith LD. Inhibition of *Clostridium botulinum* by strains of *Clostridium perfringens* isolated from soil. *Appl Microbiol* 1975;30:319–323.
63. Kelch WJ, Kerr LA, Pringle JK, et al. Fatal *Clostridium botulinum* toxicosis in eleven Holstein cattle fed round bale barley haylage. *J Vet Diagn Invest* 2000;12:453–455.
64. Sandler RJ, Rocke TE, Yuill TM. The inhibition of *Clostridium botulinum* type C by other bacteria in wetland sediments. *J Wildl Dis* 1998;34:830–833.

65. Allison MJ, Maloy SE, Matson RR. Inactivation of *Clostridium botulinum* toxin by ruminal microbes from cattle and sheep. *Appl Environ Microbiol* 1976;32:685–688.
66. Grecz N, Arvay LH. Effect of temperature on spore germination and vegetative cell growth of *Clostridium botulinum*. *Appl Environ Microbiol* 1982;43:331–337.
67. Gale P. Risks to farm animals from pathogens in composted catering waste containing meat. *Vet Rec* 2004;155:77–82.
68. Gale P. Risk assessment: use of composting and biogas treatment to dispose of catering waste containing meat. Final report to the department for environment, food and rural affairs (DEFRA). London: DEFRA, WRC-NSF Ltd, 2008.
69. Grewal SK, Rajeev S, Sreevatsan S, et al. Persistence of *Mycobacterium avium* subsp *paratuberculosis* and other zoonotic pathogens during simulated composting, manure packing, and liquid storage of dairy manure. *Appl Environ Microbiol* 2006;72:565–574.
70. Wright PE, Inglis SF, Stehman SM, et al. Reduction of selected pathogens in anaerobic digestion. Available at: [www.manuremanagement.cornell.edu/docs/](http://www.manuremanagement.cornell.edu/docs/). Accessed on Mar 17, 2008.
71. Monteith HD, Shannon EE, Derbyshire JB. The inactivation of a bovine enterovirus and a bovine parvovirus in cattle manure by anaerobic digestion, heat treatment, gamma irradiation, ensilage and composting. *J Hyg (Lond)* 1986;97:175–184.
72. Huang H, Spencer JL, Soutyrine A, et al. Evidence for degradation of abnormal prion protein in tissues from sheep with scrapie during composting. *Can J Vet Res* 2007;71:34–40.
73. Gale P, Stanfield G. Towards a quantitative risk assessment for BSE in sewage sludge. *J Appl Microbiol* 2001;91:563–569.
74. McQuiston JH, Garber LP, Porter-Spalding BA, et al. Evaluation of risk factors for the spread of low pathogenicity H7N2 avian influenza virus among commercial poultry farms. *J Am Vet Med Assoc* 2005;226:767–772.
75. ProMED 2/5/2007—anthrax, human, bovine—Australia (Victoria). Available at: [www.promedmail.org/pls/otn/f?p=2400:1202:3759577525819148:NO:F2400\\_P1202\\_CHECK\\_DISPLAY,F2400\\_P1202\\_PUB\\_MAIL\\_ID:X,36236](http://www.promedmail.org/pls/otn/f?p=2400:1202:3759577525819148:NO:F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,36236). Accessed Sept 3, 2008.
76. ProMED 9/5/2007—anthrax, bovine—Russia (Buryatia). Available at: [www.promedmail.org/pls/otn/f?p=2400:1202:1423323494241309:NO:F2400\\_P1202\\_CHECK\\_DISPLAY,F2400\\_P1202\\_PUB\\_MAIL\\_ID:X,39149](http://www.promedmail.org/pls/otn/f?p=2400:1202:1423323494241309:NO:F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,39149). Accessed Sept 3, 2008.
77. Scientific Panel on Biological Hazards of the European Food Safety Authority. Opinion of the Scientific Panel on Biological Hazards of the European Food Safety Authority on the safety vis-à-vis biological risks of biogas and compost treatment standards of animal by-products (ABP). *EFSA J* 2005;264:1–21.
78. Tonner-Klank L, Moller J, Forslund A, et al. Microbiological assessments of compost toilets: in situ measurements and laboratory studies on the survival of fecal microbial indicators using sentinel chambers. *Waste Manag* 2006;27:1144–1154.
79. Vinnerås B. Comparison of composting, storage and urea treatment for sanitising of faecal matter and manure. *Bioresour Technol* 2007;98:3317–3321.
80. Vinnerås B, Björklund A, Jönsson H. Thermal composting of faecal matter as treatment and possible disinfection method—laboratory-scale and pilot-scale studies. *Bioresour Technol* 2003;88:47–54.
81. Smårs S. An advanced experimental composting reactor for systematic simulation studies. *J Agric Eng Res* 2001;78:415–422.
82. Smårs S, Gustafsson L, Beck-Friis B, et al. Improvement of the composting time for household waste during an initial low pH phase by mesophilic temperature control. *Bioresour Technol* 2002;84:237–241.
83. Christensen KK, Carlsbaek M, Kron E. Strategies for evaluating the sanitary quality of composting. *J Appl Microbiol* 2002;92:1143–1158.
84. Pillai SD, Widmer KW, Dowd SE, et al. Occurrence of airborne bacteria and pathogen indicators during land application of sewage sludge. *Appl Environ Microbiol* 1996;62:296–299.

## Appendix

List of D and z values for selected microbial pathogens and indicators derived during evaluation for thermal resistance with regard to thermophilic composting.

Type of matrix	Pathogen	Mean D value* (min)				Mean z value (°C† [95% CI])
		55°C	60°C	65°C	70°C	
Liquid	<i>Salmonella</i> spp <sup>30</sup>	3.7	0.4	0.04	0.001	5.2 (5.1–5.3)
	<i>Salmonella</i> Senftenberg 775W <sup>30</sup>	40.8 (293 at 50°C)	5.7	NR	NR	5.8 (5.4–6.4)
	<i>Campylobacter jejuni</i> <sup>30</sup>	0.83	0.13	0.02	0.0016	6.4 (5.8–7.0)
	<i>Escherichia coli</i> <sup>30</sup>	4.43	0.65	0.09	0.006	6.0 (5.9–6.1)
	<i>Enterococcus faecium</i> <sup>30</sup>	63	19	5.8	1.08	9.6 (8.8–10.5)
	<i>Listeria monocytogenes</i> <sup>30</sup>	10.7	1.45	0.2	0.011	5.7 (5.6–5.9)
	<i>Yersinia enterocolica</i> <sup>30</sup>	2.8	0.5	0.09	0.008	6.7 (6.0–7.7)
	<i>Clostridium perfringens</i> —vegetative cells <sup>31</sup>	16.3	NR	0.9	1.3	7.8
	<i>C perfringens</i> —spores <sup>31</sup>	NR	NR	NR	(2.2 at 100°C) (34.2 at 90°C)	8.4
	<i>Clostridium botulinum</i> —spores <sup>32</sup>	NR	NR	NR	72–100	6.8–7.5
	<i>Bacillus cereus</i> —vegetative cells <sup>31</sup>	(33.2 at 50°C)	1.0	NR	0.2	6.6
	<i>B cereus</i> —spores <sup>31</sup>	NR	NR	NR	(2 at 95°C) (32 at 85°C)	8.5
	<i>Cryptosporidium</i> spp—oocysts <sup>33</sup>	NR	1.0	NR	NR	NR
	Compost	<i>Salmonella</i> spp <sup>34</sup>	30–60	15–20	NR	NR
<i>Salmonella</i> Senftenberg 775W <sup>35</sup>		89	7.5	NR	NR	NR
<i>E coli</i> <sup>34</sup>		60	15–20	NR	NR	NR
<i>Mycobacterium tuberculosis</i> <sup>34</sup>		NR	NR	15–20	20	NR
<i>Ascaris lumbricoides</i> —ova <sup>35</sup>		NR	1.7	NR	NR	NR
<i>Entamoeba histolytica</i> —cysts <sup>35</sup>		44	2.5	NR	NR	NR
Bacteriophage f2 <sup>35</sup>		267	47	NR	NR	NR
Poliovirus type 1 <sup>35</sup>		32	19	NR	NR	NR
Adenovirus 12 NIAID <sup>35</sup>		11	0.17	NR	NR	NR

\*The D value (min) is the amount of time required to cause a 10-fold (1 log<sub>10</sub>) reduction in the number of organisms (in various matrices). †The mean z value is the temperature (°C) change needed and 95% confidence interval (CI) to change the D value by a factor of 10 (ie, the slope of the thermal death-time semilog<sub>10</sub> plot).  
NR = Not reported.