ACL Complaint R5-2013-0592
Attachment A
Footnote 1 Reference

**Carcass Disposal: A Comprehensive Review**

National Agricultural Biosecurity Center Consortium
USDA APHIS Cooperative Agreement Project
Carcass Disposal Working Group

August 2004

**Chapter 1**

# Burial

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## Abbreviations

| | |
|---|---|
| AI | avian influenza |
| APHIS | USDA Animal and Plant Health Inspection Service |
| BOD | biochemical oxygen demand |
| BSE | bovine spongiform encephalopathy |
| COD | chemical oxygen demand |
| CWD | chronic wasting disease |
| CAFO | confined animal feeding operation |
| CJD | Creutzfeldt-Jakob disease |
| DEFRA | UK Department for Environment Food and Rural Affairs (formerly MAFF, UK Ministry of Agriculture, Fisheries and Food) |
| EA | UK Environment Agency |
| END | exotic Newcastle disease |
| EPA | US Environmental Protection Agency |
| FMD | foot and mouth disease |
| MAFF | UK Ministry of Agriculture, Fisheries & Food |
| MSW | municipal solid waste |
| NAO | UK NAO |
| RHD | rabbit hemorrhagic disease |
| SEAC | Spongiform Encephalopathy Advisory Committee |
| TDS | total dissolved solids |
| TOC | total organic carbon |
| Ton | US ton (2,000 lbs) |
| Tonne | Metric ton (2,204 lbs) |
| TSE | transmissible spongiform encephalopathy |
| TVOC | total volatile organic compounds |
| UK | United Kingdom |
| US | United States |
| USDA | United States Department of Agriculture |
| VOC | volatile organic compounds |
| WHO | World Health Organization |

DEFRA, 2002b). In a speech to the US Animal Health Association, Taylor (2001) indicated that "the present evidence suggests that TSE infectivity is capable of long-term survival in the general environment, but does not permit any conclusions to be drawn with regard to the maximum period that it might survive under landfill conditions." In 2003, the European Commission Scientific Steering Committee emphasized that the "extent to which [potential TSE] infectivity reduction can occur as a consequence of burial is poorly characterized" (European Commission Scientific Steering Committee, 2003). Based on this lack of understanding, along with concerns for groundwater contamination and dispersal or transmission by vectors, the committee indicated that burial of animal material which could possibly be contaminated with BSE/TSEs "poses a risk except under highly controlled conditions" (e.g., controlled landfill) (European Commission Scientific Steering Committee, 2003).

## 1.3 – Implications to the Environment

### Animal carcass decomposition

From the point at which an animal (or human) succumbs to death, degradation of bodily tissues commences, the rate of which is strongly influenced by various endogenous and environmental factors (Pounder, 1995). Soft tissue is degraded by the postmortem processes of putrefaction (anaerobic degradation) and decay (aerobic degradation) (Micozzi, 1991, p. 37). Putrefaction results in the gradual dissolution of tissues into gases, liquids, and salts as a result of the actions of bacteria and enzymes (Pounder, 1995). A corpse or carcass is degraded by microorganisms both from within (within the gastrointestinal tract) and from without (from the surrounding atmosphere or soil) (Munro, 2001, p. 7; Micozzi, 1986). Generally body fluids and soft tissues other than fat (i.e., brain, liver, kidney, muscle and muscular organs) degrade first, followed by fats, then skin, cartilage, and hair or feathers, with bones, horns, and hooves degrading most slowly (McDaniel, 1991, p. 873; Munro, 2001, p. 7).

Relative to the quantity of leachate that may be expected, it has been estimated that about 50% of the total available fluid volume would "leak out" in the first week following death, and that nearly all of the immediately available fluid would have drained from the carcass within the first two months (Munro, 2001). For example, for each mature cattle carcass, it was estimated that approximately 80 L (~21 gal) of fluid would be released in the first week postmortem, and about 160 L (~42 gal) would be released in the first two months postmortem. However, the author noted that these estimates were based on the rates of decomposition established for single non-coffined human burials, which may not accurately reflect the conditions in mass burials of livestock (Munro, 2001). Another source estimated the volume of body fluids released within two months postmortem would be approximately 16 $m^3$ (16,000 L, or ~4,230 gallons) per 1000 adult sheep, and 17 $m^3$ (17,000 L, or ~4,500 gallons) per 100 adult cows (UK Environment Agency, 2001b, p. 11).

Regarding the gaseous by-products that may be observed from the decomposition of animal carcasses, one report estimated the composition would be approximately 45% carbon dioxide, 35% methane, 10% nitrogen, with the remainder comprised of traces of other gases such as hydrogen sulfide (Munro, 2001). Although this report suggested that the methane proportion would decrease over time, with very little methane being produced after two months, a report of monitoring activities at one of the UK mass burial sites suggests that gas production, including methane, increases over time, rather than decreases (Enviros Aspinwall, 2002b).

The amount of time required for buried animal carcasses (or human corpses) to decompose depends most importantly on temperature, moisture, and burial depth, but also on soil type and drainability, species and size of carcass, humidity/aridity, rainfall, and other factors (McDaniel, 1991; Pounder, 1995; Mann, Bass, & Meadows, 1990). A human corpse left exposed to the elements can become skeletonized in a matter of two to four weeks (Mann, Bass, & Meadows, 1990; Iserson, 2001, p. 384); however, an unembalmed adult human corpse buried six feet deep in ordinary soil without a coffin requires approximately ten to twelve years or more to skeletonize (UK Environment Agency, 2002a; Pounder, 1995; Munro, 2001; Iserson, 2001). In addition to actual carcass material in a burial site, leachates or other pollutants may also persist for an extended period. Although much of the pollutant load would likely be released during the earlier stages of decomposition (i.e., during the first 1–5 years) (UK Environment Agency, 2001b; McDaniel, 1991; UK Environment Agency, 2002a; Munro, 2001), several reports suggest that mass burial sites could continue to produce both leachate and gas for as long as 20 years (UK Environment Agency, 2001b; Det Norske Veritas, 2003).

## Environmental impacts

Various works have estimated the potential environmental impacts and/or public health risks associated with animal carcass burial techniques. Several sources identify the primary environmental risk associated with burial to be the potential contamination of groundwater or surface waters with chemical products of carcass decay (McDaniel, 1991; Ryan, 1999; Crane, 1997). Freedman & Fleming (2003) stated that there "has been very little research done in the area of environmental impacts of livestock mortality burial," and concluded that there is little evidence to demonstrate that the majority of regulations and guidelines governing burial of dead stock have been based on any research findings directly related to the environmental impacts of livestock or human burials. They also conclude that further study of the environmental impacts of livestock burial is warranted.

During the 2001 outbreak of FMD in the UK, various agencies assessed the potential risks to human health associated with various methods of carcass disposal (UK Department of Health, 2001c; UK Environment Agency, 2001b). The identified potential hazards associated with burials included body fluids, chemical and biological leachate components, and hazardous gases. Further summaries of environmental impacts are outlined in investigations into the operation of various mass disposal sites (Det Norske Veritas, 2003; UK Environment Agency, 2001c).

Since precipitation amount and soil permeability are key to the rate at which contaminants are "flushed out" of burial sites, the natural attenuation properties of the surrounding soils are a primary factor determining the potential for these products of decomposition to reach groundwater sources (UK Environment Agency, 2002a). The most useful soil type for maximizing natural attenuation properties was reported to be a clay-sand mix of low porosity and small to fine grain texture (Ucisik & Rushbrook, 1998).

Glanville (1993 & 2000) evaluated the quantity and type of contaminants released from two shallow pits containing approximately 62,000 lbs of turkeys. High levels of ammonia, total dissolved solids (TDS), biochemical oxygen demand (BOD), and chloride in the monitoring well closest to the burial site (within 2 ft) were observed, and average ammonia and BOD concentrations were observed to be very high for 15 months. However, little evidence of contaminant migration was observed more than a few feet from the burial site.

The impact of dead bird disposal pits (old metal feed bins with the bottom removed, placed in the ground to serve as a disposal pit) on groundwater quality was evaluated by Ritter & Chirnside (1995 & 1990). Based on results obtained over a three-year monitoring period, they concluded that three of the six disposal pits evaluated had likely impacted groundwater quality (with nitrogen being more problematic than bacterial contamination) although probably no more so than an individual septic tank and soil absorption bed. However, they cautioned that serious groundwater contamination may occur if a large number of birds are disposed of in this manner.

In the aftermath of the 2001 UK FMD outbreak, the UK Environment Agency (2001b) published an interim assessment of the environmental impact of the outbreak. The most notable actual environmental pressures associated with burial included odor from mass burial sites and landfills, and burial of items such as machinery and building materials during the cleansing and disinfection process on farms. The interim environmental impact assessment concluded that no significant negative impacts to air quality, water quality, soil, or wildlife had occurred, nor was any evidence of harm to public health observed. Monitoring results of groundwater, leachate, and landfill gas at the mass disposal sites indicated no cause for concern (UK Public Health Laboratory Service, 2001c).

### Monitoring programs

Following the disposal activities of the 2001 FMD outbreak, the UK Department of Health outlined environmental monitoring regimes focused on the key issues of human health, air quality, water supplies, and the food chain (UK Department of Health, 2001b; UK Public Health Laboratory Service); these programs might serve as models for monitoring programs in the aftermath of an animal disease eradication effort. The UK programs included monitoring of public drinking water supplies, private water supplies, leachate (levels, composition, and migration), and surveillance of human illness (such as gastrointestinal infections). Chemical parameters and indicators were reported to likely be better than microbiological parameters for demonstrating contamination of private water supplies with leachate from an animal burial pit, but testing for both was recommended. It was recommended that at-risk private water supplies should be tested for chloride, ammonium, nitrate, conductivity, coliforms, and *E. coli*. Because baseline data with which to compare would likely not exist, caution in interpretation of results was stressed (i.e., increased levels of an analyte may not necessarily indicate contamination by a disposal site; other sources may be involved) (UK Public Health Laboratory Service).

# Section 2 – Historical Use

This chapter primarily addresses three burial techniques, namely trench burial, landfill, and mass burial sites. This section contains a brief overview of the historical use of these methods for disposal of animal carcasses.

One burial technique not addressed in this report is that of a "burial pit," which consists of a hole dug into the earth, the sides of which may be lined with concrete, metal, or wood. The bottom of the pit is left exposed to the earth below, and the top is closed with a tight-fitting cover or lid. In the past, this technique was used extensively by the poultry industry as a convenient means of disposing of daily mortalities. However, this technique is not specifically addressed in this chapter, as it is not well-suited to the disposal of large quantities of material, and the use of such pits is generally being phased out due to environmental concerns.

The general frequency with which burial techniques, and other methods, are used by various livestock or food animal operations to dispose of daily mortalities is outlined in Table 1. The information contained in this table was summarized from various reports prepared under the National Animal Health Monitoring System of the Veterinary Services Division of the United States Department of Agriculture (USDA), Animal & Plant Health Inspection Service (APHIS). While these values may not reflect the situation that may occur during an animal health emergency, they provide some insight into the disposal methods used on an ongoing basis to dispose of daily production mortalities.

## 2.1 – Trench Burial

### Background

Trench burial has been used throughout history as a method of carcass disposal. For animal disease eradication efforts in the US, trench burial has traditionally been a commonly used, and, in some cases even a preferred, disposal option (USDA, 1981; USDA, APHIS, 1978). In spite of its logistical and economic advantages, concerns about possible effects on the environment and subsequently public health have resulted in a less favorable standing for this method, especially when large numbers of carcasses may be involved.

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# On-Farm Mortality Composting of Livestock Carcasses

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Livestock mortality is an issue faced by every livestock farming operation, both large and small. For many producers, carcass disposal options are limited, can be costly, and may temporarily disturb the land needed for grazing. Improper disposal of dead animal carcasses and the resulting leachate (carcass fluids) can negatively impact surface water and groundwater quality. If the animal died of an infectious disease, pathogenic bacteria and viruses may be present inside the carcass, thereby increasing risk of disease transmission. Additionally, state regulations exist regarding the proper disposal of livestock mortalities. Oklahoma State University (OSU) Extension fact sheet BAE-1748 provides information about these regulations and the state approved methods for livestock carcass disposal, which include: burial, rendering, incineration, composting and landfills. Table 1 lists the criteria that determine the acceptability and desirability for each carcass disposal method.

**Table 1. Goals of carcass disposal.**

| |
|---|
| Fulfills regulations |
| Creates postive public perception |
| Reduces disease transmission |
| Promotes environmental sustainability |
| Produces beneficial by-product |
| Economical |
| Practical |

## Composting: Simple Solution for Large and Small Farms

One state approved procedure that livestock producers may not be familiar with is composting. Properly managed composting fulfills each of the desired goals established in Table 1. By definition, composting is a controlled biological decomposition process that converts organic matter into a stable, humus-like product. OSU fact sheet BAE-1744 describes the basic process of composting.

While backyard composting systems have a well-blended mixture of components (carbon and nitrogen), which results in a rapid compost cycle, livestock composting is a slower process. Composting livestock carcasses is characterized by the break down of a large centralized nitrogen source (carcass) that is surrounded by a carbon source (bulking agent). This system requires an initial breakdown of the soft tissues on the exterior of the carcass, followed by thorough mixing to promote an ideal blend of carbon and nitrogen for effective composting.

Four key aspects of composting include:

1) Carbon to Nitrogen (C:N) ratio
2) Oxygen
3) Moisture content
4) Temperature

These four aspects determine the efficiency of the livestock composting system and are controlled by the bulking agent. The correct bulking agent provides the proper C:N ratio needed to successfully compost the carcass while ensuring adequate oxygen levels, maintaining ideal moisture and promoting heat retention. The bulking agent also contains any leachate and odors produced during the process, therefore acting as a filter between the carcass and the environment. Microorganisms will degrade the carcass leaving only a few small bone fragments, which are brittle and break easily. This valuable by-product can then be land applied as a fertilizer source, adding nutrients and organic matter to the soil, or reused for additional carcasses. The high temperatures (130 F to 150 F) achieved through proper composting will destroy most pathogens and weed seeds. Table 2 illustrates mortality losses of livestock in Oklahoma and the potential impact of mortality compost nutrients if land applied.

**Table 2. Annual Oklahoma cattle and calf death loss and carcass nutrient data.**

| | *Cattle* | *Calves* | *Total* |
|---|---|---|---|
| OK Inv. (# head) | 2.1 million | 3.3 million | 5.4 million |
| Death Loss (%) | 2.1§ | 6.4§ | 4.8 |
| Mortalities (# head) | 44,100 | 212,850 | 256,950 |
| Average Wt. (lbs) | 1246‡ | 460Ω | - |
| Avg. Mortality (lbs) | 54.9 million | 97.9 million | 152.8 million |
| Projected Carcass C (lbs/head) | 180 | 66.5 | - |
| Projected Carcass N (lbs/head) | 36 | 13.3 | - |
| Total Projected C (lbs) | 7.9 million | 14.1 million | 22.1 million |
| Total Projected N (lbs) | 1.5 million | 2.8 million | 4.4 million |
| Projected Value of N† | $556,500 | $990,817 | $1.5 million |

† Based on a conservative value of $0.35 per pound of N as Urea.
* This does not include the added value of increased organic matter, Ca, P, K or other nutrients.
§ National Death Loss Survey, USDA. 1996-2005.
‡ Livestock Marketing Information Center, LMIC. 1999-2008.
Ω National Stocker Survey, BEEF. 2008.

## Steps to Composting Livestock Mortalities

### Site Selection

One of the more important aspects of macro-composting is careful site selection. An ideal location for an exposed compost pile is an elevated site sufficiently distant from bodies of water and neighboring properties. The site should be located in an area that does not pose a risk to surface water or groundwater contamination. Figure 1 illustrates the proper placement of a compost site with respect to distance from water bodies.

Exposed sites tend to get adequate airflow, but can be affected by local climate and weather patterns. Excessively dry or wet conditions may decrease compost efficiency. Picking an elevated site is desirable to reduce the risk of perched water tables and groundwater contamination. Site slope should be kept to a minimum to discourage excessive erosion around the pile and possible runoff.

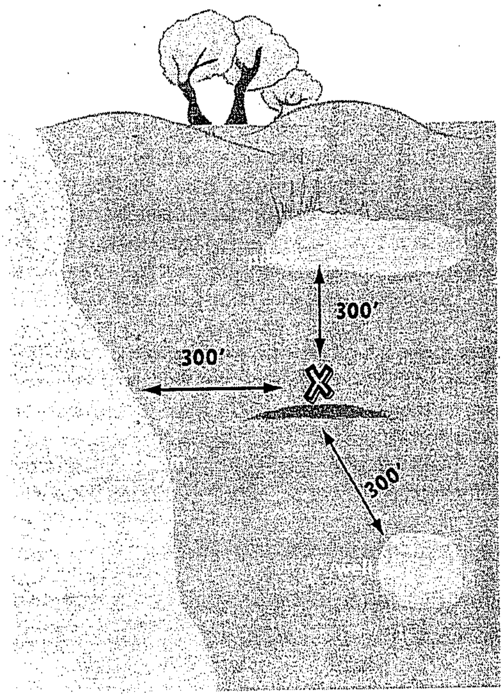


**Figure 1. Site selection for composting.**

### Building a Compost Barrier

A barrier wall or fence is optional but does present some advantages during the composting process. At the very least in their design, barriers should guard against physical intrusion from livestock and predators, while restricting movement of carbon material. Barriers do not need to be elaborate or expensive. One inexpensive, yet effective approach is to construct a bin using 4 feet high field fence supported by four steel t-posts (Figure 2). Barriers can also be constructed permanently with a concrete floor, treated wood walls and a metal roof. The design is the producers' choice, but it should be based on the number of animals to compost and the investment level desired. Unrestricted piles must be sloped from the base to the peak much like a pyramid. However, using a barrier will contain the bulking agent depth in a smaller footprint, reducing the amount of C material required. This is an economical benefit that can recoup the cost of barrier construction (See Figure 3). Other options include old pallets, round hay bales or cattle panels.

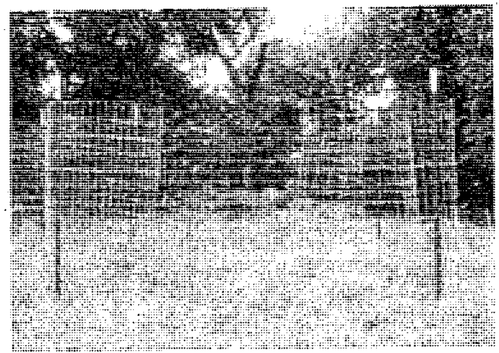

**Figure 2. Bin constructed from steel t-posts and net wire.**

### Compost Bin Foundation

The bin foundation is not as important to the process of composting as to the ease of maintenance on the piles. Foundations range from the ground itself, to pallets, gravel and concrete. Again, the option is completely up to the producer. Using bare soil is acceptable if proper site selection was implemented, and the carbon pad beneath the carcass is of adequate depth to contain carcass leachate. Concrete, on the other hand, makes a very nice permanent foundation that is easy to clean and maintain. Consideration should be given to the method of aeration, since foundations of gravel, pallets, etc. may prove to be difficult to mix and turn with equipment.

### Carbon Source

A C source, preferably with a high C:N, must be chosen before construction can begin. Table 3 lists common C:N ratios for various compost materials. The C source is the "filter" between the carcass and the environment. Therefore, choosing the right carbon source is vital to the success of the livestock composting system. Sources of carbon should be easily obtainable in the local area. Any woody, stemmy or fibrous material usually makes a good C source. Common options are wood chips, shavings or sawdust, hay, straw, corn stubble, chipped tree limbs, rice hulls, etc. These all have moderate to high C:N ratios that when added to the nitrogen rich carcass promote efficient composting.

Select C sources that are fine to medium in porosity for optimum efficiency. Particle size is important in regulating air flow. Too much airflow can cool and dry out a pile, while too little airflow can inhibit oxygen availability, both of which can cause microbial activity to slow. Coarse sources, such as long-stemmed wheat straw, have poor thermal efficiency and allow excessive moisture to escape the pile. When dealing with these types of C sources, such as hay or straw bales, prior weathering or grinding is recommended.

Another recommended option is to incorporate manure with the selected C source. This addition can help to reduce the porosity and further homogenize the C:N. Note: If poultry litter is used in the compost mix, Oklahoma state law requires that it be covered overhead or surrounded by a compacted soil berm to prevent litter movement.

**Table 3. Common compost materials.**

| *Compost Material* | *C:N* |
|---|---|
| Sawdust[1] | 442:1 |
| Straw-wheat[1] | 127:1 |
| Rice hulls[1] | 121:1 |
| Straw-general[1] | 80:1 |
| Corn stalks[1] | 60-73:1 |
| Finished compost[1] | 30-50:1 |
| Hay-general[1] | 15-32:1 |
| Horse manure-general[1] | 30:1 |
| Cattle manure[1] | 19:1 |
| Grass clippings[1] | 17:1 |
| Sheep manure[1] | 16:1 |
| Turkey litter[1] | 16:1 |
| Broiler litter[1] | 14:1 |
| Swine manure[2] | 14:1 |
| Cottonseed meal[1] | 7:1 |
| Soybean meal[1] | 4-6:1 |
| Animal carcass[2] | 5:1 |

[1]On-Farm Composting Handbook, Agriculture, and Engineering Service, NRAES-54, Natural Resource, Ithaca, New York.
[2]Compost Materials, 1996 EBAE172-93, North Carolina Cooperative Extension Service, Raleigh, North Carolina.

**Building a Pad**

For single calves up to 800 lbs, a foundation and pad built 8 ft² is sufficient. If there is a possibility of composting mature cows, this footprint should be increased to 10 ft². The C pad is a very important feature in the composting process. Therefore, certain design rules should be adhered to. The roles of the C pad include providing thermal insulation from the foundation, a "filter" to absorb and contain carcass leachate while preventing its entry into the environment, and a carbon source for composting occurring at the bottom of the carcass. Therefore, the ideal pad should be 18 inches in depth for fine particle sources (shavings, rice hulls, etc.) and 24 inches for coarse particle sources (straw, corn stalks, etc.).

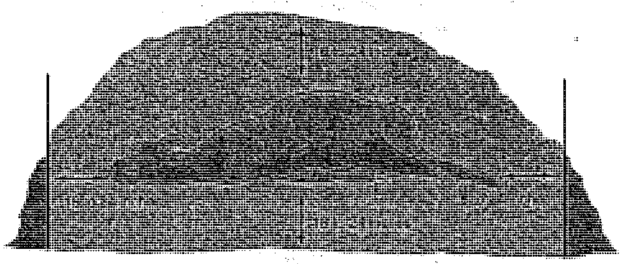

**Figure 3. Proper pad design and carcass placement when composting livestock, as well as the potential reduction in required bulking agent when utilizing a barrier.**

## Carcass Placement & Water Requirements

The carcass should be placed on the center of the pad. The head and legs may need to be tied with baling twine to avoid obtrusion through the pile (Figure 4). Once the carcass is placed on the pad, an incision should be made along the abdominal cavity, puncturing and deflating the rumen. If this procedure is not performed, the rumen can swell and rupture causing a portion of the pile to collapse. A further option is lancing the large muscle groups and opening the body cavity, exposing the internal organs. This procedure allows microbial access to the interior of the carcass and upon addition of the C source, speeds up decomposition.

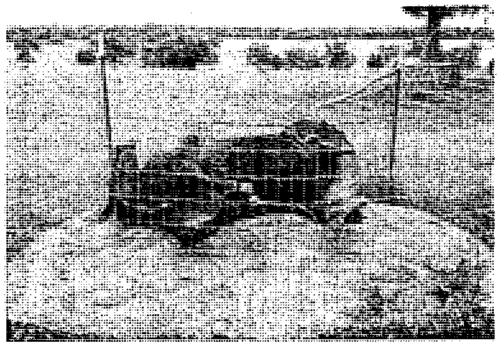

**Figure 4. Carcass placed in center of the pad with head and legs tied to avoid obtrusion.**

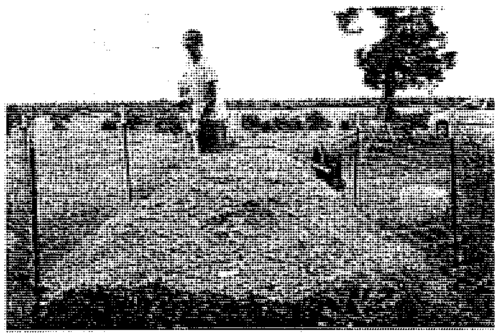

**Figure 5. Carcass surrounded with at least 18 inches of bulking agent.**

The bulking agent used for the pad and cover should contain approximately 50 percent moisture by weight. If using a dry bulking agent, water should be added. Pond water works well because it contains an abundance of microorganisms. As a rule of thumb, the bulking agent should be moist to the touch, but you should not be able to squeeze out drops of water. If piles are too dry, microorganisms may die or remain inactive, resulting in cool piles with slow decomposition rates. If conditions within the pile are too wet, airflow is limited and oxygen availability is reduced, potentially leading to foul odors.

**Adding the Carbon Cover**

The C cover performs similar functions as the pad. As such, maintaining a thickness of at least 18 inches for fine particle sources and 24 inches for coarse particle sources will meet the desired goals. These goals include reducing odors to minimal or nonexistent levels, providing sufficient C for composting above and later inside the carcass, and in some cases providing a "cap" to shed excess rainfall. Yet, the cover plays the largest role in the efficiency of the decomposition process. Since heat rises and also carries moisture, the cover insulates the core from temperature loss and resultant moisture loss. Maintaining an adequate C cover thickness of ideal porosity is the key to optimum livestock composting. In rainy areas, piles should be designed with steep crowns to shed rainfall.

## 10 Steps for Proper Large Animal Carcass Composting

1. Construct barrier and base at chosen site.
2. Prepare carbon pad at least 18 inches deep.
3. Place animal in center, ensuring the carcass is at least 24 inches from the pad edge.
   a. If necessary, use baling twine to hold legs and head in position.
4. Lance rumen to deflate gas buildup.
5. Add water to carcass and pad until C source is damp but not wet.
6. Finish with at least 18 inches of carbon cover over the entire carcass.
7. For exposed piles, form a steep peak to shed excess water.
8. After 75 days, the first heat cycle should be finished. Turn the pile while mixing and aerating the carbon material. Large bones should remain in the core of the pile.
9. After 150 days, the second heat cycle is nearing completion. Turn the pile again to further cure.
   a. Remaining bone fragments should be brittle but can be placed in the next pile for complete decomposition.
10. Land apply the material as you would fertilizer or use to compost additional carcasses.

### Managing the Pile

Internal pile temperature should be monitored using a long-stem thermometer throughout the composting process (Figure 6). The pile should begin to heat within the first day or two, transitioning from the moderate to high temperature phase. Internal pile temperatures should reach more than 130 F. This is due to the metabolic energy produced by the active microorganisms. Over time, the microorganisms consume the available soft tissue, carbon and oxygen within the pile. As microbial activity decreases, the temperature within the pile begins to drop, thus entering the cooling phase. This process may take one month to five months depending on climate, C source, etc. Opening the pile too soon may lead to an undesirable release of foul odors. Once the temperature drops 30 F below maximum temp, or below 110 F, it is time to turn the pile.

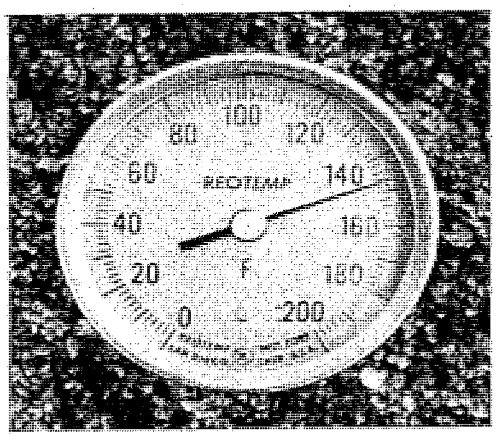


Figure 6. Long-stem thermometer used to monitor temperature.

**Estimates per 100 lb cattle carcass**

| | | |
|---|---|---|
| Water | → | 7 gals |
| Carbon | → | 15 lbs |
| Nitrogen | → | 3 lbs |
| Phosphorus | → | 0.7 lb |

**Additional Requirements per 100 lb cattle carcass**

| | | |
|---|---|---|
| Carbon Source | → | 1 cubic yard |
| Water | → | 1 gal to 2 gals |

Using a front end loader, turn the pile while mixing the remaining carcass and bulking agent. Introduce new oxygen by cascading the bulking agent from the loader into the pile (Figure 7). Assess moisture levels to determine if water should be added. Make sure all carcass parts are once again covered with adequate bulking agent depth. A similar rise and fall in temperature should occur as active microorganisms decompose bones. If properly managed, the pile should begin to enter the curing process. At this point, the producer can decide to land apply the compost as a valuable fertilizer source or aerate the pile again to further cure. The finished compost can also be recycled to seed the new pile with microorganisms or used as a bulking agent with new mortalities. Any remaining large bones should be brittle and break easily (Figure 8). If desired, they can be added to another compost pile for further decomposition.

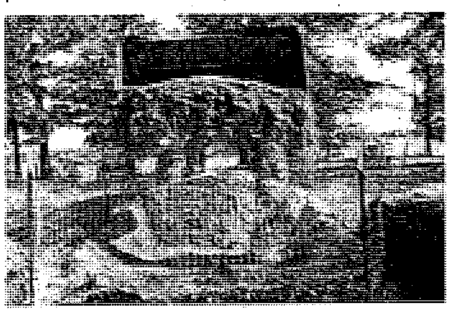

**Figure 7. Turning the pile with a front-end loader.**

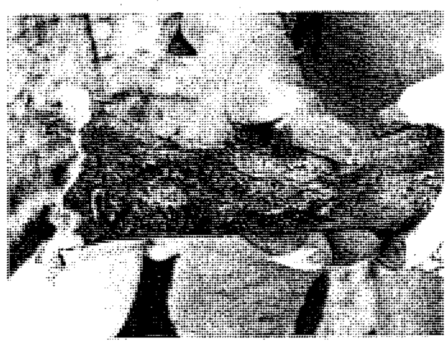

**Figure 8. A hollow, brittle femur bone following 150 days of composting with pine shavings and poultry litter mixture.**

Since all producers may not have access to a long-stem thermometer, it is beneficial to have a timeline for correct pile management. OSU research on livestock composting has shown that by 75 days after adding a carcass, the first heat cycle is ending, and it is recommended to turn the pile. Following proper aeration, the second heat cycle will be completed in an additional 75 days. Final turning and aeration can be performed at this time. Compost piles should not be turned any sooner than this, as these guidelines are minimum time requirements. Other factors that can lengthen turn times include cool winter temperatures, which slow the decomposition process, and larger carcasses, which require a longer decomposition time.

An OSU field study conducted in southeast Oklahoma, near Stigler, compared three bulking agents for composting stocker calf carcasses. The treatments consisted of pine shavings (S), a 50:50 mixture of pine shavings and poultry litter (S&L), and hay (H). Each treatment was replicated four times, and piles were turned on days 75 and 150. The findings indicated that S, S&L and H treatments were all effective at decomposing stocker calf soft tissue over a 150 day period. Shavings and S&L treatments formed a humus-like product and were more effective at decomposing bones when compared to the H treatment. Additionally, S and S&L treatments maintained sufficient temperatures required for effective pathogen reduction or elimination, while H treatments lost heat and moisture due to a higher porosity (Figure 9).

**Windrowing for Multiple Mortalities**

When composting multiple livestock mortalities, establishing windows of bulking agent is recommended due to the increased quantity of carcasses. Site selection and pad width and depth should follow previous bin construction recommendations. However, pad length and carcass placement differ slightly. The back of one carcass may rest on the legs of the adjacent carcass as illustrated in Figure 10. Assure 24 inches of space from the carcass to the edge of the pad. Providing a fence structure may not be feasible. Therefore, properly covering the carcass with 24 inches of bulking agent is essential to prevent scavenger invasion. The length of the pile is dependent on the number of mortalities to be composted. Pile management should follow similar recommendations as previously outlined for bin structures.

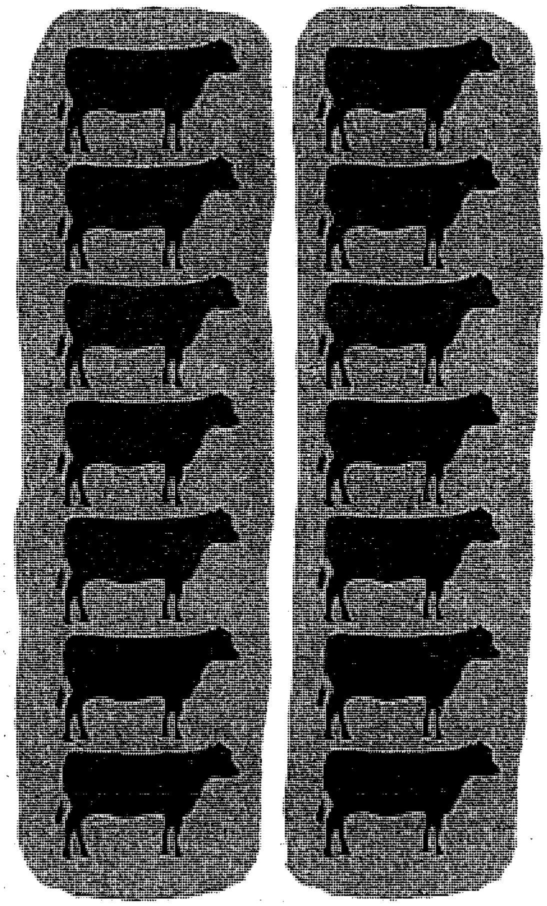

**Figure 10. Windrow composting for multiple mortalities.**

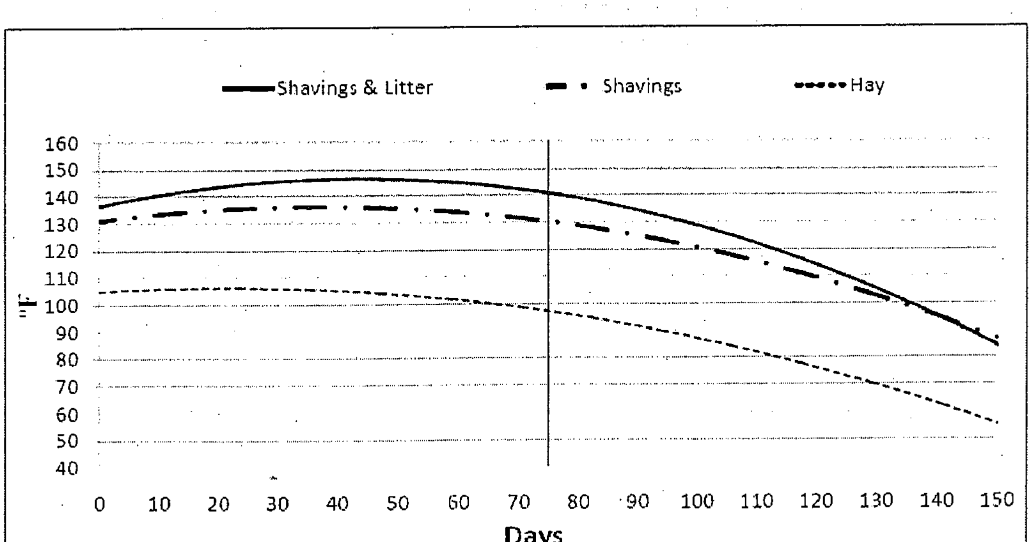


**Figure 9. Temperature variation over time of pine shavings and poultry litter mixture (S&L), pine shavings (S) and hay (H) compost piles each containing a stocker calf.**

## Summary

Sustainable livestock production requires proper management of on-farm mortalities regardless of farm size. These methods should adequately dispose of animal carcasses without negatively affecting the environment, while also remaining economical to the producer. When properly managed, composting livestock mortalities is a safe, effective option for producers to consider, while producing a valuable soil amendment.

For additional information on composting livestock carcasses, refer to these resources:

Auvermann, B., S. Mukhtar, and K. Heflin. 2006. Composting Large Animal Carcasses. Texas Cooperative Extension Publication E-422. College Station, TX. Available at: http://tammi.tamu.edu/largecarcassE-422.pdf

Bonhotal, J., L. Telega, and J. Petzen. 2002. Natural Rendering: Composting Livestock Mortality and Butcher Waste. Cornell Waste Management Institute. Ithaca, NY. Available at: http://compost.css.cornell.edu/naturalrenderingFS.pdf

Morse, D.E. 2006. Composting Animal Mortalities. Minnesota Department of Agriculture. St. Paul, MN. Available at: http://www.mda.state.mn.us/news/publications/animals/compostguide.pdf

VanDevender, K., and J. Pennington. 2004. Organic Burial Composting of Cattle Mortality. University of Arkansas Cooperative Extension Publication FSA-1044. Little Rock, AR. Available at: http://www.uaex.edu/Other_Areas/publications/PDF/FSA-1044.pdf



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# *Planning Considerations for Dairy Cattle Disposal by On-farm Burial*

27.

By Tom Glanville, Department of Agricultural & Biosystems Engineering

While a major portion of livestock mortalities are handled by the rendering industry, the number of rendering plants has declined in recent years. Some dairy operators say they no longer can obtain rendering service in their area, others are faced with higher rendering fees or less frequent service, and renderers have stopped accepting cattle more than 30 of age since they require special processing before they can be used in animal feed. As a result, a growing number of producers are considering on-farm disposal. This article looks at some of the pros and cons of on-farm burial as an alternative to rendering.

One of the first questions that livestock producers often ask is how the costs of burial compare with those for rendering. Since there is no quarterly bill to pay for on-farm burial, the true costs — of the land for the burial site itself; for the time and labor needed to excavate and close trenches; and the capital and operating costs of the equipment needed for burial — can be difficult to assess. Since rendering service fees include all costs associated with that option, a fair cost comparison should include all costs associated with alternative disposal methods. When all costs associated with burial are carefully reported, some studies have actually shown that burial costs exceed those for rendering. A 2001 Iowa State University survey of mortality disposal costs reported by 300 Iowa swine producers, for example, showed that the average total cost of burial was more than twice that of rendering.

Beyond the initial concerns related to cost, are additional questions concerning convenience, operational flexibility, and special facilities or equipment that may not be a normal part of livestock production. When properly planned and managed, on-farm burial offers the flexibility of being able to handle mortalities of any size. Weather permitting, burial is reasonably convenient, and required facilities and equipment—a backhoe for trench or pit excavation and backfilling, and a sufficient amount of well-drained land area for a burial site—are often part of existing operations. Excavation and backfilling can be difficult when the ground is frozen, but this is typically overcome by opening a sufficient length of trench during warm weather to meet anticipated burial needs when the soil is frozen.

The primary disadvantage of burial is its potential to contaminate soil, shallow groundwater, or nearby streams that derive their dry weather flow from shallow groundwater. The burial-related pollutant of greatest concern is nitrogen which can be released as both ammonia, or nitrite and nitrate. Total ammonia-nitrogen concentrations of only 1-2 milligrams per liter (mg/L) can create chronic toxicity problems for young fish, and drinking water containing nitrate-nitrogen concentrations greater than 10 mg/L poses health threats to human infants.

As every crop and livestock producer who has ever developed a nutrient management plan knows, when nitrogen application rates significantly exceed agronomic rates for crop production, the potential for groundwater pollution increases. Unless livestock burial rates are purposely limited, the amount of nitrogen contained in carcasses can easily exceed agronomic rates. A 1200 pound cow carcass contains about 24 pounds of nitrogen that will be released into the soil as the carcasses degrade. That's not a lot of N only if a single cow is buried occasionally, but if animals are buried frequently in the same area year-after-year on a continuing basis, can become equivalent to application of more than 30,000 lbs of N per acre. Stacking of carcasses in a deep pit or trench can cause even higher rates. Even if subsurface decomposition takes 10-20 years this equates to average N releases well in excess of typical agronomic rates. Since carcasses are often buried four or more feet below ground this puts the carcass nitrogen below the root zone for many crops, reducing the potential for beneficial uptake, and increasing the risk that the N will ultimately leach into shallow groundwater.

To avoid the nitrogen pollution potential described above, the weight of carcasses buried in a given area should be limited. To accomplish this Iowa DNR rules limit routine on-farm burial to seven cattle, 44 swine, 73 sheep or lambs, or 400 poultry carcasses **on any given acre per year**. All other species are limited to 2 carcasses per acre.

When catastrophes cause sudden loss of large numbers of animals, higher loading rates than those listed above are permitted by Iowa DNR on a case-by-case basis if local geology and other conditions are judged to be such that local water resources will not be seriously impaired. Be sure to contact Iowa DNR (emergency phone number is 515/281-8694) for a ruling on emergency burial sites before proceeding with disposal. Due to the potential long-term environmental consequences of a large burial site, Iowa DNR may require the land owner to file an affidavit with the county assessor documenting the existence of the site on the deed to your property.

For planning purposes, the Iowa DNR interactive *Livestock Burial Zones* map, which can be accessed on the World Wide Web at http://www.iowadnr.gov/mapping/maps/livestock_burial_zones.html is a useful tool for identifying areas on your property that are suitable for burial of large quantities of carcasses. Using the interactive map, you can view maps and aerial photos of your property that identify potential problem locations for mass burial based on Iowa DNR's geographic information system database.

To further limit the potential for damage to valuable water resources and property, IDNR rules also require that burial sites be located outside of wetlands, floodplains, and shoreline areas. Maximum allowable burial depth is six feet, burial must be at least two feet above the highest seasonal groundwater elevation, and carcasses must be covered with at least 30 inches of soil. Required horizontal setbacks are **at least**: 100 feet from a private well, stream, lake, or pond; 200 feet from a public well; 50 feet from property lines; and 500 feet from a residence.

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ACL Complaint R5-2013-0592
Attachment A
Footnote 4 Reference

# Water Quality Impacts of Burying Livestock Mortalities

by:
**Rachel Freedman and Ron Fleming**
Ridgetown College - University of Guelph

**August, 2003**

presented to the:

**Livestock Mortality Recycling Project Steering Committee**

Ridgetown College

UNIVERSITY of GUELPH

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## Introduction

Mortality losses are a normal part of livestock and poultry production. Producers may have losses due to disease, accidents, or inter-animal competition. It is the responsibility of the producer to dispose of these mortalities in an acceptable manner. Livestock and poultry producing regions in Canada and many other industrialized countries have put into place regulations governing acceptable disposal methods for these on-farm mortalities. In Ontario, the Dead Animal Disposal Act outlines three legal disposal methods for dead cattle, swine, sheep, goats, and horses:

a) pickup by a provincially licensed collector;
b) composting under 60 cm (2 feet) of organic substrate, such as sawdust or straw; and
c) burying under 60 cm (2 feet) of soil and away from all waterways (Koebel 2001).

Using the services of a provincially licensed collector seems to be the preferred method of disposal in Ontario. However, some regions of the province, particularly the northwest, do not have access to the services of a licensed collector. Recently, we have seen a withdrawal or reduction of these services in other areas due to concerns over the spread of livestock diseases in rendered animal products (i.e. Bovine Spongiform Encephalopathy). In those cases where a licensed collector is not available, producers must rely on either composting or burial.

Composting offers a smart solution to carcass disposal problems. The finished compost can be used as a nutrient-rich organic soil amendment. However, composting not only requires a proper facility, but also a certain amount of ongoing monitoring and care. In addition, larger animals such as cattle are more difficult to compost. This leaves many time-constrained producers with burial as their only viable option.

Buried livestock mortalities undergo a decomposition process. During this process, nutrients, pathogens, and other components of the animal carcass are released into the environment. As these substances enter the surrounding soil, they may be broken down, transformed, lost to the air, or otherwise immobilized so that they pose no environmental threat. However, there is a possibility that some constituents may eventually contaminate soil, groundwater, and surface water. It is unlikely that a single carcass could cause major contamination. However, in light of the trend toward large-scale livestock production practices, there is concern over the numbers of mortalities that could be buried.

## Objectives

In light of the trends in the livestock industry and the potential for negative impacts on the environment, a few questions concerning the burial of mortalities have arisen. Are current regulations and guidelines regarding livestock mortality disposal (and burial) meeting the needs of today's producers? Are the regulations meeting the needs of the environment? This report is an attempt to examine the current state of knowledge in the area of livestock carcass burial and the potential for water quality impacts. Specific objectives are:

1. Determine the current state of knowledge of water quality (and other environmental) impacts of livestock mortality burial.
2. Recommend what, if any, new information is needed, relevant to the needs of Ontario livestock producers.

3. Prepare updated recommendations, if needed, for farmers who want to bury livestock mortalities.

## Numbers of Mortalities

As mentioned earlier, mortality losses are a normal part of livestock and poultry production. There is variability in the numbers of these losses from one farm to another and across livestock species. Table 1 contains estimates of these mortality losses for Ontario farms. These numbers put into perspective the scope of the issue. The greatest mass of mortalities is in the form of chickens - laying hens and broilers. Cattle represent the next greatest mass, followed by swine and turkeys.

**Table 1: Total Species Numbers, Estimated Average Mortality Rates and Weights For Different Livestock and Poultry Species in Ontario**

| Type of Livestock or Poultry | Total Species Numbers in Ontario | Average Mortality Rate† | Average Weight kg (lbs)† | Approximate Annual Mass of Mortalities in Ontario (t) |
|---|---|---|---|---|
| Cattle | 2,160,000* | 3.6% | 341 (750) | 26,516 |
| Horses, Ponies | 83,337** | 3.6% | 341 (750) | 1,023 |
| Sheep | 280,000* | 6.2% | 24.7 (77.4) | 429 |
| Goats | 62,310** | 6.2% | 24.7 (77.4) | 95 |
| Swine | 3,714,700* | 6.3% | 70 (153) | 16,382 |
| Chickens | 199,876,000* | 7.1% | 2.5 (5.8) | 35,478 |
| Turkeys | 8,422,000* | 6.7% | 11.1 (24.4) | 6,263 |
| Bison | 3,755** | 1.6% | 359 (791) | 22 |
| Elk | 5,902** | 2.5% | 188 (414) | 28 |
| Ranched Deer | 14,464** | 2.8% | 68 (149) | 28 |
| Mink | 84,800*** | 4.5% | 2 (4) | 8 |
| Foxes | 560*** | 7.5% | 7 (16) | 0.3 |
| | | | **Total mass (t)** | **86,272.3** |

*Ontario Ministry of Agriculture and Food, 2003.
**Statistics Canada, 2002.
***Statistics Canada, 2003.
†Morris, J., Koebel, G., 2003.

# Environmental Impacts of Livestock Mortality Burial

There has been very little research done in the area of environmental impacts of livestock mortality burial. An exhaustive search of published information turned up only a small number of studies. (Note: the search was limited to reports written in English, or those with English abstracts.) This lack of scientific information has been confirmed by Tom Glanville, one of the few researchers to study the issue (Glanville 2003). Research has been mainly focussed on poultry mortality pits and their effects on the surrounding environment. However, we should expect to see more interest as concerns with Foot and Mouth Disease and Bovine Spongiform Encephalopathy persist.

Following are summaries of the few reports that have been written on the subject:

1. In a presentation to the American Society of Agricultural Engineers (ASAE), Glanville (2000) reported on the impact of livestock burial on shallow groundwater quality. He noted that proper disposal of livestock mortalities can be more difficult than manure management because animal carcasses are not easily stored for long periods of time and cannot be spread on cropland. Biosecurity and environmental impacts must be considered when disposing of livestock mortalities. In order to study the characteristic types, concentrations, and duration of release of contaminants from on-farm burial, the Iowa Department of Natural Resources (IDNR) funded two case studies.

The first case study examined two 1.8 m deep pits containing 28,400 kg of turkey carcasses that had been buried one year prior to the beginning of the study. The site was located in poorly drained soil with moderately-slow permeability. The seasonal high water table could be found at depths of 0.3 to 0.9 m. Twelve monitoring wells were used to define contaminant movement and background water quality. Groundwater samples were collected monthly for a period of 15 months, and again at 20 months and 40 months.

Case study number two sampled two 1.2 m deep trenches spaced 2.4 m apart in well-drained, moderately permeable soil. At this site the seasonal high water table could be found at a depth greater than 1.8 m. This site was specially constructed at the Iowa State University Agricultural Engineering research farm. Each trench was loaded with six 11.3 - 13.6 kg swine carcasses spaced evenly along the trench bottom. The mass of carcasses in each trench was considered a reasonable loading rate according to IDNR rules. One of the trenches was lined with PVC sheeting and ten centimetres of pea gravel. A PVC pipe was buried vertically at one end of the trench and outfitted with a sump pump so that monthly samples of leachate could be obtained. The leachate was measured to examine the mass, concentration, and duration of decay products. Eight monitoring wells were placed around the trenches to monitor groundwater.

Elevated levels of Biochemical Oxygen Demand (BOD), Ammonia-Nitrogen ($NH_4$-N), Total Dissolved Solids (TDS), and Chloride (Cl) were commonly found within or very near the burial trenches. Although chloride concentrations were generally lower than the other contaminants, elevated chloride levels are generally the best indicator of burial-related groundwater contamination. Localized contamination may persist for a decade or more in wet soil with a high seasonal water table and low groundwater flow velocity. Even in lightly loaded burial trenches constructed in well drained soil, complete decay may take two years or more. Neither of these experiments showed burial-related contamination more than a metre or two from the pits. In cases where groundwater velocities are higher, or where vertical groundwater movement occurs, leachate from burial sites may pose a higher contamination risk to groundwater.

2. The microbiology of graves is a relatively unknown subject. Hopkins et al (2000) were able to use a forensic experiment involving the burial of pigs to gain knowledge in this area. This experiment was originally meant to supply information on the decomposition of human bodies. However, the use of pig carcasses provided a useful study into the decomposition of livestock mortalities. Three pig carcasses (four to five months of age) were buried within three hours of death, under ten centimetres of clay-based soil in a hornbeam dominated woodland in late December. At 430 days (roughly 14 months) after burial, soil samples were taken from each of the graves and control samples were collected one metre from each grave. At this time, it was noted that the pigs' bodies had lost their integrity and the graves contained mixtures of decaying remains and soil. The results of this experiment showed elevated ammonium concentrations, biomass, and respiratory activity, which all indicate that decomposition was still taking place at the time of sampling.

3. Myers (1998) looked at the impact of poultry mortality pits on groundwater quality in Georgia. There were a number of methods allowed for carcass disposal in Georgia. Burial was the most common method of disposal, but farmers required a permit for their disposal pit and were subject to regular checks of the pits. The covered pits used for disposal were dug into the ground but left unlined, so leachate from the decomposing carcasses could travel through the soil. The leachate could contain nitrates, microbes, and other potential water contaminants. Four areas were chosen to be sampled, one in clay soil and the others in sand soil. Older mortality pits were sampled using electromagnetic survey, water quality monitoring, lysimeters and test wells. Results of the leaching study were not available at the time the 1998 report was published. However, the final report should be available soon (Myers 2003).

4. Ritter et al (1988) examined the impact of dead bird disposal on groundwater quality. They monitored groundwater quality around six disposal pits in Delaware. Producers in Delaware were using open-bottomed pits for their day-to-day mortality disposal. These pits are not strictly the same as burial pits, though there are some similarities. Most of these pits were located in sandy soils with high seasonal water tables. The potential for pollution of groundwater is high with this method of disposal. After selecting the sites, two to three monitoring wells were placed around each pit to a depth of 4.5 metres. Ammonia concentrations were high in two of the wells. Three of the disposal pits caused an increase in ammonia concentrations in the groundwater. Total dissolved solids concentrations were high in all monitoring wells for most dates. Bacterial contamination of groundwater by the disposal pits was low.

5. In a related study, Ritter and Chirnside (1995) looked at the impact of dead bird disposal pits on groundwater quality on the Delmarva Peninsula. They reported these additional discoveries:

- nitrogen is a greater problem than bacterial contamination,
- serious contamination may occur if large numbers of birds are added to the pit,
- abandoned disposal pits should be pumped out and filled with soil to minimize their impact on groundwater quality,
- subsurface disposal of dead birds should be regulated,

- only certain types of disposal pits (i.e. concrete tanks) should be allowed, and
- permits should be issued for disposal sites meeting minimum standards (i.e. dealing with soil-type, water table depth, etc.).

6. Crane (1997) discussed the potential environmental impacts of the disposal of livestock carcasses in the United Kingdom. This paper did not report on a research project - rather it was a discussion of existing practices. Crane concluded that all animal carcasses have the potential to cause environmental damage. Pets and animals from commercial sites were disposed of as controlled waste, and were therefore subject to the stringent Waste Management Licensing Regulations. However, agricultural waste, including carcasses, was not considered "controlled waste" and was not subject to stringent regulations. The acceptable methods for animal carcass disposal were by: a treatment/processing plant, burning, or burial.

According to guidelines: carcasses should be buried deep enough so that carnivorous animals cannot dig them up, and the carcasses should be buried in a type of ground that prevents water table contamination. Carcasses can also be buried at a licensed or unlicensed landfill site. The advantage of a licensed site is that the issue of groundwater protection has been addressed in the licensing process. The Ministry of Agriculture, Fisheries, and Food (MAFF) Code of Practice for carcass disposal advised contacting the Environment Agency if unsure of the suitability of a burial site. It also included guidelines governing distances to water tables, drinking water, etc. However, the guidelines did not address the issue of the monitoring of burials. According to this report, it is unlikely that carcass burial has resulted in any major groundwater contamination. The greatest risks are related to the chemical products of decomposition. However, there is no evidence that significant harm has occurred due to burials. While individual carcass burial is not a cause for concern, as carcass numbers increase so does the need for site assessment.

## Environmental Impacts of Human Burial

Because so little information was available on burial of livestock mortalities, a review of studies on human burial was carried out. There has been a limited amount of scientific research regarding water quality impacts of human burial. Most of the work has been done in recent years.

**Decomposition of the Body - Processes**

Human bodies undergo the same processes of decomposition as animal carcasses. As previously stated, nutrients, pathogens and other components of the body are released into the environment during the process.

A body's decomposition is directly related to soil condition and above-ground temperature. As depth increases, decomposition rates are slowed. As above-ground temperature increases, decomposition increases (Spongberg and Becks 2000). Although the source of contamination is finite (at some point in time the body will have completely broken down), the length of time that organic matter is released into the environment is dependent on a number of factors. Body size, temperature, and precipitation can all affect the decomposition rate (Spongberg and Becks 2000).

The decay of a human body can also be influenced by: a) the features of the remains, b) the

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Attachment A
Footnote 5 Reference

# Use and Environmental Occurrence of Antibiotics in Freestall Dairy Farms with Manured Forage Fields

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Environmental releases of antibiotics from concentrated animal feeding operations (CAFOs) are of increasing regulatory concern. This study investigates the use and occurrence of antibiotics in dairy CAFOs and their potential transport into first-encountered groundwater. On two dairies we conducted four seasonal sampling campaigns, each across 13 animal production and waste management systems and associated environmental pathways: application to animals, excretion to surfaces, manure collection systems, soils, and shallow groundwater. Concentrations of antibiotics were determined using on line solid phase extraction (OLSPE) and liquid chromatography-tandem mass spectrometry (LC/MS/MS) with electrospray ionization (ESI) for water samples, and accelerated solvent extraction (ASE) LC/MS/MS with ESI for solid samples. A variety of antibiotics were applied at both farms leading to antibiotics excretion of several hundred grams per farm per day. Sulfonamides, tetracyclines, and their epimers/isomers, and lincomycin were most frequently detected. Yet, despite decades of use, antibiotic occurrence appeared constrained to within farm boundaries. The most frequent antibiotic detections were associated with lagoons, hospital pens, and calf hutches. When detected below ground, tetracyclines were mainly found in soils, whereas sulfonamides were found in shallow groundwater reflecting key differences in their physicochemical properties. In manure lagoons, 10 compounds were detected including tetracyclines and trimethoprim. Of these 10, sulfadimethoxine, sulfamethazine, and lincomycin were found in shallow groundwater directly downgradient from the lagoons. Antibiotics were sporadically detected in field surface samples on fields with manure applications, but not in underlying sandy soils. Sulfadimethoxine and sulfamethazine were detected in shallow groundwater near field flood irrigation gates, but at highly attenuated levels.

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## Introduction

Pharmaceuticals of both human and veterinary origins have been widely detected in various environmental matrices including surface water, groundwater, soils, and sediments (*1, 2*). The use of veterinary antibiotics in concentrated animal feeding operations (CAFOs) is a growing concern as a significant source of contamination (*3*). Antibiotics are used in livestock production to prevent and treat diseases, promote growth, and improve productivity (*4*). In the U.S., 12.6 thousand metric tons of antibiotics were sold for animal use in 2007, 13% of which were administered to promote growth and efficiency (*5*). Antibiotics and their metabolites are excreted in feces and urine, and escape containment during normal waste management operation and surface runoff (*6*). Once antibiotics are released from CAFOs, they may affect terrestrial and aquatic organisms (*7–9*) and may lead to the development of antibiotic-resistant strains of microorganisms (*10–12*).

California is the largest U.S. producer of milk and cheese with 1.8 million milking cows, making freestall dairies the state's most prevalent CAFO industry. Most of California's dairies are located in the San Joaquin Valley (*13*), a topographically flat region overlying predominantly alluvial and fluvial unconsolidated sediments with some areas of shallow water table, which are particularly vulnerable to groundwater contamination. Little is known about the potential for antibiotic migration from freestall dairy operations into groundwater (runoff to streams is prohibited). Dairies administer significantly less antibiotics per unit animal weight than other CAFO industries in accordance with the grade "A" Pasteurized Milk Ordinance, which prohibits administration of most antibiotics to lactating cows (*14*) except monensin, an ionophore used as a feed additive to increase milk production (*15*). However, antibiotics are prophylactically used on calves, heifers, and dry cows, raising concerns of significant antibiotic loading to the environment, especially in regions with high concentration of dairy farms.

This is the first study to comprehensively evaluate the fate of antibiotics in dairy operations, from administration to excretion, waste collection, land application, and potential soil–water transport under relatively vulnerable groundwater conditions. At two farm study sites, the major dairy management units were sampled, where each is characterized by specific antibiotic uses or waste management operations. Analysis of soil and water samples permitted us to assess the potential for off-site migration of antibiotics, and to identify environmental conditions that promote retention or on-site degradation of antibiotics.

## Materials and Methods

The research dairies are located on the distal alluvial fans of the Stanislaus River and the Tuolomne River just east of the northern San Joaquin Valley trough. Groundwater levels at the study sites range from 2–5 m below ground surface. The dominant soil texture is sandy loam. The shallow saturated and overlying unsaturated zone consists of predominantly

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silty fine sand with intercalated, discontinuous clayey silt. The average regional groundwater flow rate is $5 \times 10^{-7}$ m s$^{-1}$ (*16*). Monitoring wells, located throughout the dairies (Supporting Information (SI) Figure S1), are screened from 3 to 10 m below ground surface. Shallow groundwater samples have a composite age ranging from weeks to approximately two years. The associated upgradient source area is 150 m to several hundred meters in length and a few to tens of meters in width (*16, 17*).

During the study, dairy I housed 1450 lactating cows, 1400 heifers, and 250 dry cows. Dairy II consisted of 1340 lactating cows, 1240 heifers, and 470 dry cows. Before weaning, calves are kept in individual hutches. Calf hutches are located on a raised structure at Dairy I. Its floor is flushed with clean groundwater three times per day. Calf hutches at Dairy II sit on bare ground. Heifers are kept in separate freestalls grouped by age. Adult cows are kept in freestalls and have access to adjacent exercise yards (corrals) between feedings. Freestall flush-lanes are lined with concrete, and are flushed three to four times per day with recycled lagoon water to collect excrement. Solid waste is separated from the waste stream, and recycled as bedding material in freestalls and exercise yards after drying. Wastewater is returned to the lagoon. Offsite runoff is not permitted. Corral surface runoff and dairy wash-water are collected in the lagoon. At both dairies, the lagoons were constructed over 30 years ago with a soil linear containing 10% clay. Liquid manure and unused solids are applied as fertilizer to surrounding forage fields, which typically comprise over 75% of the total farm area (*15*). Similar modern freestall dairy operations can be found worldwide.

For this study, the following dairy management units were sampled: calf hutches, hospital pens, liquid manure storage lagoons, solid and liquid manure applied fields, and corrals and freestalls for heifers, for milking cows, and for dry cows (*15*), (SI Figure S1). Environmental pathways that may allow antibiotics to be transported into groundwater include leakage from lagoons, leaching of manure applied to fields, and leaching from animal housing areas.

Samples were collected from surfaces (loose soil/litter materials), soil (<30 cm depth), wastewater, and shallow groundwater in four campaigns over 18 months representing fall, winter, spring, and summer climate conditions and operations status. Surface samples in the dairy production area were taken from bedding materials composed of dried solid manure, and those in the field were taken from loose surface soil. Concentrations of antibiotics were determined using online solid phase extraction (OLSPE) and liquid chromatography-tandem mass spectrometry (LC/MS/MS) with electrospray ionization (ESI) for water samples (*18*). Solid samples were extracted using the accelerated solvent extraction (ASE) method described in McKinney et al. (*19*) and analyzed by direct aqueous injection of the solid sample extracts using a Shimadzu Prominence LC and API 5000 tandem MS (Columbia, MD) in multiple-reaction-monitoring (MRM) mode with ESI and positive-negative ion switching (see SI for details).

## Results and Discussion

**Pharmaceutical Usage.** A wide variety of pharmaceuticals were used in the study dairies, with total farm application rates varying from 0.02 to 660 g d$^{-1}$ according to interviews with the participating farmers (Table 1). Substantial differences were observed between the types and quantities of pharmaceuticals applied at the two dairies. At both dairies, penicillin procaine G, monensin, and acetylsalicylic acid (aspirin) had the highest use (several hundred g d$^{-1}$), followed by ampicillin, ceftiofur, sulfonamides, and tetracyclines (several tens of g d$^{-1}$). Assuming no loss of antibiotics in the waste collection system by degradation or sorption, estimated worst-case antibiotic concentrations in lagoon water range from tens of ng L$^{-1}$ to hundreds of $\mu$g L$^{-1}$ (Table 1, details of the estimate are in SI Table S3).

**Occurrence and Transport: Waste Management Systems.** *Lagoon and Flush-Lane Water.* Sulfonamides and trimethoprim, tetracyclines and their epimers/isomers, and lincomycin were detected frequently in lagoon and flush-lane water samples with concentrations ranging from 0.012 to 267 $\mu$g L$^{-1}$ (Table 2). Epimers/isomers of chlortetracycline were detected although the parent chlortetracycline was not present, whereas both tetracycline and its epimer were found when the concentration of tetracycline was close to 0.1 $\mu$g L$^{-1}$. Importantly, all antibiotics on the analytical schedule known to be administered at the farms were detected in lagoon and flush-lane water. The presence of the complete suite of administered antibiotics in the dairy waste system is consistent with the broad spectrum of human-applied pharmaceuticals found in municipal wastewater systems (*20*). It is possible that other pharmaceuticals used at these dairies but not on the analytical schedule were also present.

For the few studies which report antibiotics in dairy lagoon water, detections included tetracycline, iso-chlortetracycline, epi-iso-chlortetracycline, and lincomycin ranging from 0.01 to 7.7 $\mu$g L$^{-1}$ (*3, 21*), similar to this study. The spectrum of antibiotics reported in swine lagoons is similar to those found in our study, with chlortetracycline, iso-chlortetracycline, epi-iso-chlortetracycline, lincomycin, oxytetracycline, sulfamethazine, tetracycline, sulfathiazole, tylosin, erythromycin-$H_2O$, and penicillin G frequently reported at concentrations ranging from high ng L$^{-1}$ to low mg L$^{-1}$ (*1, 3, 22, 23*). However, the lagoon water concentrations of antibiotics detected in this study were in the ng L$^{-1}$ to low $\mu$g L$^{-1}$ range, lower than those reported in swine lagoons. Lower antibiotics amounts administered, higher water use, and larger lagoon size, among other factors, may explain this difference. In particular, swine often receive antibiotics as feed additives (*24*), whereas antibiotic use in feed additives of dairy farms is limited. The spectrum of compounds detected in this study is similar to that in swine lagoons, suggesting similar transport processes and persistence in the waste-stream.

Observed concentrations of tetracycline, epi-tetracycline, chlortetracycline, iso-chlortetracycline, epi-so-chlortetracycline, lincomycin, and trimethoprim were at least 1 order of magnitude smaller than the theoretical maximum concentration estimated for lagoon water (Table 1, 2). In at least one sampling event, sulfonamide concentrations in the Dairy I lagoon were on the same order of magnitude as the theoretical maximum, suggesting that the attenuation of sulfonamides in the wastewater system may not always be significant.

The observed concentration variability was high (Table 2), possibly due to intermittent use of antibiotics. Freestall flush-lane water, recycled from the lagoons, was sampled to capture added antibiotics from feces and urine collected during flushing. However, the range of concentrations detected in flush-lane water was comparable to those in lagoon water samples. Hence, the concentration increase in flush water due to addition of antibiotics from fresh feces and urine was much smaller than lagoon water concentrations. Also, detected compounds apparently do not substantially degrade within the waste storage and recycling system.

The calf hutches flush used fresh groundwater rather than recycled lagoon water. It provided a better measure of antibiotics excretion. Relatively high concentrations of sulfamethazine, sulfamethoxazole, oxytetracycline, and trimethoprim, were detected there, reflecting the intensive use of antibiotics on calves and the significant contribution of fresh urine and feces to antibiotics in wastewater.

*Lagoon Sediments.* Sediments from a lagoon were collected to assess sediment-solution partitioning during percolation. Sulfamethazine (36 $\mu$g kg$^{-1}$), total chlortetracycline

**TABLE 1. Pharmaceuticals Used in the Study Dairy Farms and Theoretical Maximum Concentrations in Lagoon Water[a]**

| class | | use g $d^{-1}$ | | theoretical maximum concentration in lagoon[b] $\mu g\ L^{-1}$ | |
|---|---|---|---|---|---|
| antibiotics | compound | Dairy I | Dairy II | Dairy I | Dairy II |
| aminoglycoside | dihydrostreptomycin | 17.1 | | 13 | |
| beta-lactam | amoxicillin | 0.05 | | 0.1 | |
| | ampicillin | | 31.3 | | 76 |
| | cloxacillin | 0.13 | | 0.1 | |
| | penicillin procaine G | 660.0 | 56.0 | 750 | 135 |
| cephalosporin | ceftiofur | 14.6 | 10.8 | 16 | 25 |
| | cephapirin | 0.1 | | 0.1 | |
| chloramphenicol derivative | florfenicol | 2.5 | 1.9 | 2 | 3 |
| lincosamide | **lincomycin** | 6.0 | 5.5 | 8 | 15 |
| | pirlimycin | | 0.03 | | 0.1 |
| macrolide | **tylosin** | | | | |
| | **erythromycin** | | | | |
| sulfonamides | **sulfadimethoxine** | 24.3 | | 5 | |
| | **sulfamethazine** | 8.8 | 10.8 | 1 | 3 |
| | **sulfamethoxazole** | 8.8 | | 3 | |
| tetracycline | **oxytetracycline** | 7.1 | 2.6 | 8 | 6 |
| | **tetracycline** | | | | |
| | **chlortetracycline** | | | | |
| other | **trimethoprim** | 1.8 | | 1 | |
| ionophore | lasalocid | 4.1 | | 3 | |
| | monensin | 388.8 | 31.0 | 246 | 42 |
| quinolone | decoquinate | | 7.2 | | 17 |
| anti-inflammatory non steroidal | acetylsalicylic acid | 445.7 | 369.1 | 68 | 119 |
| | flunixin meglumine | | 2.1 | | 2 |
| steroidal | isoflupredone acetate | 0.02 | | 0.03 | |
| | dexamethasone | 0.10 | 0.24 | 0.1 | 0.5 |
| diuretic | furosemide | 1.8 | 0.09 | 1 | 0.1 |

[a] Compounds in bold were analyzed in this study. The pharmaceuticals were identified and total masses used were obtained through interviews with the dairy owners and veterinary staff, and by examining the dairy's purchase receipts over the preceding 6- to 9-month period. The theoretical maximum is the total mass of pharmaceutical excreted divided by the lagoon volume. The details of the estimate are in the SI. Theoretical maximum concentration in lagoon = use × excretion rate × retention time/lagoon volume. Lagoon volumes are $6.66 \times 10^4\ m^3$ (Dairy I) and $8.98 \times 10^4\ m^3$ (Dairy II). Retention times (84.1 d (Dairy I) and 241 d (Dairy II)) are calculated using the daily water use estimate proposed by Meyer et al. (55). See SI for details on excretion rate. [b] Theoretical maximum concentrations were estimated assuming no attenuation.

(176 $\mu g\ kg^{-1}$), oxytetracycline (109 $\mu g\ kg^{-1}$), and tetracycline (42 $\mu g\ kg^{-1}$) were detected in a lagoon sediment sample (Table 2). The apparent distribution coefficients ($K_{d\ app}$) between the lagoon water and the sediment for sulfamethazine and oxytetracycline were 8.3 and 351 L $kg^{-1}$, respectively. The $K_{d\ app}$ value of oxytetracycline was somewhat greater than the reported $K_d$ values of 77.6 L $kg^{-1}$ in swine manure (25). Compared to the $K_d$ values in soils or soil constituents, $K_{d\ app}$ of sulfamethazine is greater than the reported range (0.6−3.1 L $kg^{-1}$), and $K_{d\ app}$ of oxytetracycline is within the reported range (0.3−3020 L $kg^{-1}$) (26, 27). The $K_{d\ app}$ value is subject to variations in sorbent and aqueous phase properties. In the lagoon water/sediment system, where pH is often near or above $pK_{a2}$ of tetracyclines and sulfonamides, zwitterionic or anionic species are dominant for tetracyclines, and neutral and anionic species are dominant for sulfonamides, which will result in a decrease of the $K_d$ values.

We are not aware of previous studies on antibiotics in CAFO lagoon sediments. Our data suggest that these sediments play a significant role as a sink/source of antibiotics leached by percolating lagoon water (17). Further, some farms apply lagoon sediments to their fields as soil amendments (28).

*Lagoon-Impacted Groundwater.* Shallow groundwater samples were collected 10 m downgradient of the dairy lagoons ("lagoon wells") to assess antibiotics in anoxic lagoon leakage plumes in shallow groundwater (16). Of the 10 compounds that were detected in lagoon water, only sulfadimethoxine, sulfamethazine, and lincomycin were detected in groundwater. Seven compounds present in lagoon water were attenuated to levels below the detection limit - sulfamethoxazole and trimethoprim likely due to biodegradation (29, 30), tetracyclines likely due to sorption and abiotic degradation (31, 32). Lagoon well samples at Dairy I showed higher concentrations ranging from 0.033 to 0.13 $\mu g\ L^{-1}$ for sulfadimethoxine, and 1.1 to 3.6 $\mu g\ L^{-1}$ for sulfamethazine, consistent with higher concentrations of sulfadimethoxine and sulfamethazine in lagoon water at Dairy I.

Elsewhere, concentrations of sulfadimethoxine (0.076−0.22 $\mu g\ L^{-1}$), sulfamethazine (0.046−0.067, up to 0.16 $\mu g\ L^{-1}$),

**TABLE 2. Antibiotics Concentrations in Wastewater Collection/Treatment System and Associated Shallow Groundwater[a]**

| dairy | sample | date sampled | sulfadimethoxine | sulfamethazine | sulfamethoxazole | *iso-chlortetracycline | *epi-iso-chlortetracycline | total chlortetracycline | oxytetracycline | tetracycline | *epi-tetracycline | lincomycin | trimethoprim |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | μg L$^{-1}$ | | | | | | |
| 1. water samples | | | | | | | | | | | | | |
| (1) wastewater | | | | | | | | | | | | | |
| Dairy I | lagoon water | October, 06 | 0.040 | 6.0 | 0.88 | – | – | NA | 0.093 | – | – | – | – |
| | | April, 07 | 11 | 14 | – | 1.5 | 1.0 | NA | – | 0.020 | – | – | – |
| | | September,07 | 9.0 | 4.4 | 4.9 | – | – | NA | 0.31 | – | – | – | 0.024 |
| | | January, 08 | 4.1 | 8.6 | 0.43 | 0.99 | 0.68 | NA | 0.66 | 0.11 | 0.38 | – | – |
| | flush-lane water | October, 06 | – | 5.5 | 2.0 | – | – | NA | – | – | – | – | – |
| | | April, 07 | 3.4 | 5.7 | – | 1.2 | 0.77 | NA | – | 0.14 | 0.022 | – | – |
| | | September, 07 | 0.62 | 0.26 | 0.69 | – | – | NA | – | – | – | – | – |
| | | January, 08 | 7.5 | 8.9 | 0.19 | 0.28 | 0.19 | NA | 0.090 | 0.028 | – | – | – |
| | calf hutches flush water | October, 06 | – | 15 | 19 | – | – | NA | – | – | – | – | – |
| | | May, 07 | – | – | 2.0 | – | – | NA | 0.076 | – | – | – | 0.23 |
| | | September, 07 | – | 0.63 | 1.5 | 0.017 | – | NA | 0.80 | – | – | – | 0.035 |
| | | January, 08 | NC | NC | NC | NC | NC | NA | NC | NC | NC | NC | NC |
| Dairy II | lagoon water | October, 06 | 0.25 | – | – | – | – | NA | 0.19 | 0.087 | – | 0.054 | – |
| | | April, 07 | 0.56 | – | – | – | – | NA | 0.10 | 0.074 | – | 0.044 | – |
| | | September, 07 | 0.030 | – | – | – | – | NA | – | – | – | 0.012 | – |
| | | January, 08 | 0.77 | 0.61 | – | 0.12 | 0.083 | NA | 0.18 | 0.095 | 0.035 | – | – |
| | flush-lane water | October, 06 | 0.19 | – | – | – | – | NA | 0.12 | 0.060 | – | 0.035 | – |
| | | April, 07 | 0.25 | – | – | – | – | NA | 0.19 | 0.14 | 0.089 | 0.061 | – |
| | | September, 07 | 0.035 | – | – | – | – | NA | – | – | – | – | – |
| | | January, 08 | – | – | 1.7 | 252 | 267 | NA | 0.52 | 3.1 | 2.8 | – | 2.3 |
| (2) lagoon well groundwater | | | | | | | | | | | | | |
| Dairy I | well no. 3 | October, 06 | NC | NC | – | – | – | NA | – | – | – | NC | – |
| | | April, 07 | 0.098 | 2.8 | – | – | – | NA | – | – | – | 0.23 | – |
| | | September, 07 | 0.13 | 3.6 | – | – | – | NA | – | – | – | – | – |
| | | January, 08 | 0.033 | 1.1 | – | – | – | NA | – | – | – | 0.082 | – |
| Dairy II | well no. 3 | October, 06 | 0.017 | 0.29 | – | – | – | NA | – | – | – | – | – |
| | | April, 07 | – | – | – | – | – | NA | – | – | – | 1.9 | – |
| | | September, 07 | – | – | – | – | – | NA | – | – | – | 1.1 | – |
| | | January, 08 | NC | NC | – | – | – | NA | – | – | – | NC | – |
| (3) manure-treated field groundwater | | | | | | | | | | | | | |
| Dairy I | well no. 7 | October, 06 | 0.005 | 0.037 | – | – | – | NA | – | – | – | – | – |
| | | April, 07 | – | – | – | – | – | NA | – | – | – | – | – |
| | | September, 07 | 0.010 | 0.052 | – | – | – | NA | – | – | – | – | – |
| | | January, 08 | – | 0.029 | – | – | – | NA | – | – | – | – | – |
| | well no. 9 | October, 06 | 0.006 | 0.044 | – | – | – | NA | – | – | – | – | – |
| | | April, 07 | – | 0.060 | – | – | – | NA | – | – | – | – | – |
| | | September, 07 | – | 0.061 | – | – | – | NA | – | – | – | – | – |
| | | January, 08 | – | 0.11 | – | – | – | NA | – | – | – | – | – |
| | well no. 11 | October, 06 | – | – | – | – | – | NA | – | – | – | – | – |
| | | April, 07 | – | – | – | – | – | NA | – | – | – | – | – |
| | | September, 07 | – | – | – | – | – | NA | – | – | – | – | – |
| | | January, 08 | – | 0.007 | – | – | – | NA | – | – | – | – | – |

**TABLE 2. Continued**

| dairy | sample | date sampled | sulfadimethoxine | sulfamethazine | sulfamethoxazole | *iso-chlortetracycline | *epi-iso-chlortetracycline | total chlortetracycline | oxytetracycline | tetracycline | *epi-tetracycline | lincomycin | trimethoprim |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $\mu g\ kg^{-1}$ | | | | | | |
| | 2. solid samples | | | | | | | | | | | | |
| Dairy I | lagoon sediment | August, 07 | – | 36 | – | NA | NA | 176 | 109 | 42 | – | – | – |
| | manure-treated field | | | | | | | | | | | | |
| Dairy II | surface sample | October, 06 | – | – | 6.2 | NA | NA | – | 25 | 105 | 163 | – | – |
| | | April, 07 | – | – | – | NA | NA | – | – | – | – | – | – |
| | | September, 07 | – | – | – | NA | NA | – | – | 8.8 | – | – | – |

[a] Units and wells not identified had no detectable concentrations over the course of the study. *: degradation product, –: below detection, NA: not analyzed, NC: not collected. Below detection: Dairy I: groundwater: well no. 12, surface samples: manured field, soil samples: manured field. Dairy II: soil: manured field. Below detection in water samples: Carbamazapine, Azithromycin, Erythromycin, *Erythromycin-$H_2O$, Roxithromycin, Tylosin, Virginiamycin, Ciprofloxacin, Lomefloxacin, Norfloxacin, Ofloxacin, Sarafloxacin, Enrofloxacin, Sulfachloropyridazine, Sulfadiazine, Sulfathiazole, Chlorotetracycline, *Epi-chlorotetracycline, Doxycyline, *Epi-oxytetracycline, Chloramphenicol, Ormetoprim. Below detection in solid samples: Carbamazapine, total Erythromycin A, Roxithromycin, Tylosin, Virginiamycin, Ciprofloxacin, Lomefloxacin, Norfloxacin, Ofloxacin, Sarafloxacin, Enrofloxacin, Sulfachloropyridazine, Sulfadiazine, Sulfathiazole, Doxycyline, Chloramphenicol, Ormetoprim, Trimethoprim.

sulfamethoxazole (up to 0.47 $\mu g\ L^{-1}$), and lincomycin (1.4 $\mu g\ L^{-1}$) have been reported at similar concentrations in groundwater impacted by agriculture (*33*), beef feedlots (*34*), and swine lagoons (*1*). In our study, sulfamethazine concentrations were higher than previously reported, while sulfamethoxazole was below the detection limit.

Interestingly, lincomycin was found in groundwater at Dairy I even though it was not found in lagoon water. Also, it was found in groundwater at Dairy II at higher concentrations than in lagoon water. This may reflect historic use of lincomycin as shallow groundwater is up to two years old (*16, 17*). In addition, the mode of administration of lincomycin is unique in that it is topically applied as powder in bandage on infected hooves during dry periods, whereas other antibiotics are administered systemically through injection, orally, or intramammary. The bandages should be removed after 2–5 days, but in practice they may be left to fall off, leaving the lincomycin powders remaining in the discarded bandage on the ground (*35*). With dry cows housed near the lagoon wells at both of the dairies, it is possible that lincomycin leached from the corral area. The persistence of lincomycin in this study is consistent with its known chemical (*32, 36*), photochemical (*36*) and microbial (*37*) stability.

*Surface and Soil Samples in Manure-Applied Fields.* Forage-field applications of lagoon water and manure solids represent a potential pathway for off-site migration of antibiotics into groundwater driven by recharge from irrigation or precipitation. Surface samples from fields on Dairy II contained sulfamethoxazole (6.2 $\mu g\ kg^{-1}$), oxytetracycline (25 $\mu g\ kg^{-1}$), tetracycline (8.8–105 $\mu g\ kg^{-1}$), and epi-tetracycline (163 $\mu g\ kg^{-1}$), providing evidence of environmental persistence. However, no antibiotics were detected in the manure-treated field surface samples at Dairy I, or in underlying soil samples (<30 cm depth) at either dairy, suggesting surface processes can be effective at attenuating these compounds to levels below detection. There were no detections in surface and soil samples from control fields without manure applications.

*Groundwater Underneath Manure-Treated Fields.* Sulfadimethoxine and sulfamethazine were detected in monitoring wells next to a field that received lagoon water at Dairy I, despite the fact that no antibiotics were detected in surface or soil samples. At Dairy I, sulfamethazine was detected consistently at field wells nos. 7 and 9 at concentrations ranging from 0.029 to 0.11 $\mu g\ L^{-1}$, and sporadically at well no. 11. These wells are located proximal to outlet valves of the lagoon-water flood irrigation system, where infiltration rates into soils maybe higher than elsewhere in the field. Detection of sulfadimethoxine was less frequent and close to the detection limit (0.005 $\mu g\ L^{-1}$) at nos. 7 and 9. Persistence of sulfamethazine may be attributed to the lack of anaerobic degradability (*30*). There were no detections at wells located distant from the flood irrigation outlet (no. 12 at Dairy I). Thus it appears that sulfonamides in applied lagoon water are readily transported into shallow groundwater, but do not persist in soil or at the land surface. Tetracyclines, on the other hand, are more strongly sorbed and persist at the soil surface where they are degraded. No antibiotics were detected in shallow groundwater from control wells not affected by dairy activities (Dairy I: well no. 10, Dairy II: well no. 6).

Our findings are consistent with previous studies that have observed tetracyclines in shallow soil layers and sulfonamides in leachate and groundwater. Sulfonamides weakly sorb to soils, with $K_d$ values in the range of $10^0$ to $10^1$ L $kg^{-1}$ (*26, 27, 38, 39*), whereas tetracyclines show higher sorption, with $K_d$ values from $10^2$ to $10^6$ L $kg^{-1}$ (*26, 27, 40*). One of the reasons for this difference is that tetracyclines intercalate between swelling clay layers while sulfonamides do not (*41, 42*). As a result sulfonamides persist in groundwater (*43, 44*), while tetracyclines persist in soil (*2, 43, 45–47*).

**TABLE 3. Antibiotics Concentrations in Dairy Production Areas and Associated Shallow Groundwater[a]**

| dairy | sample | date sampled | tylosin | sulfadimethoxine | sulfamethazine | sulfamethoxazole | oxytetracycline | tetracycline | *epi-tetracyucline | total chlortetracyclines | total erythromycin A | lincomycin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1. goundwater samples, dairy production area | | | | | | $\mu$g L$^{-1}$ | | | | | |
| Dairy I | well no. 4 | October, 06 | – | – | – | – | – | – | – | NA | NA | – |
| | | April, 07 | – | – | – | – | – | – | – | NA | NA | – |
| | | September, 07 | – | 0.005 | – | – | – | – | – | NA | NA | – |
| | | January, 08 | – | – | – | – | – | – | – | NA | NA | – |
| Dairy II | well no. 1 | October, 06 | 0.025 | – | 0.14 | – | – | – | – | NA | NA | – |
| | | April, 07 | NC | NC | NC | NC | NC | NC | NC | NA | NA | NC |
| | | September, 07 | – | – | 0.088 | – | – | – | – | NA | NA | – |
| | | January, 08 | – | – | 0.069 | – | – | – | – | NA | NA | – |
| | 2. solid samples (1) surface samples | | | | | | $\mu$g kg$^{-1}$ | | | | | |
| Dairy I | lactating cow freestall | October, 06 | – | – | – | – | – | – | – | – | – | – |
| | | April, 07 | – | – | – | – | – | – | – | – | – | – |
| | | September, 07 | – | – | – | – | 40 | 5.6 | – | 9.7 | – | – |
| | lactating cow | October, 06 | – | – | – | – | – | – | – | – | >1000 | – |
| | exercise yard | April, 07 | – | – | – | – | – | – | – | – | – | – |
| | | September, 07 | – | – | 7.5 | – | – | – | – | – | – | – |
| | hospital pen | October, 06 | – | 6.9 | – | – | – | 73 | – | – | – | – |
| | | April, 07 | – | 248 | – | – | – | 50 | – | – | – | – |
| | | September, 07 | – | 457 | – | – | 11 | 46 | – | – | – | – |
| | heifer exercise yard | October, 06 | – | – | – | – | – | – | – | – | – | – |
| | | May,07 | – | – | – | – | – | – | – | – | – | – |
| | | September, 07 | – | 6.4 | – | – | 24 | – | – | – | – | – |
| Dairy II | lactating cow | October, 06 | – | – | – | – | 7.9 | – | – | – | – | – |
| | freestall | April, 07 | – | – | – | – | – | – | – | – | – | – |
| | | September, 07 | – | – | – | – | – | – | – | – | – | – |
| | lactating cow | October, 06 | – | – | 10 | 36 | – | – | – | – | – | – |
| | exercise yard | April, 07 | – | – | – | – | – | – | – | – | – | – |
| | | September, 07 | – | – | – | – | – | – | – | – | – | – |
| | hospital pen | October, 06 | – | 5.8 | – | – | 18 | 19 | – | – | – | – |
| | | April, 07 | – | – | – | – | – | 6.2 | 11 | – | – | – |
| | | September, 07 | – | 43 | – | – | – | – | – | – | – | – |
| | heifer exercise yard | October, 06 | – | – | – | – | – | – | – | – | – | – |
| | | May,07 | – | – | – | – | – | – | – | – | – | – |
| | | September, 07 | – | – | – | – | 188 | – | – | – | – | – |
| | calf hutches | October, 06 | – | – | – | – | – | – | – | – | – | – |
| | | May,07 | – | – | – | – | 556 | – | – | – | – | – |
| | | September, 07 | – | – | – | – | – | – | – | – | – | – |

**TABLE 3. Continued**

| dairy | sample | date sampled | tylosin | sulfadimethoxine | sulfamethazine | sulfamethoxazole | oxytetracycline | tetracycline | *epi-tetracycline | total chlortetracyclines | total erythromycin A | lincomycin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (2) Soil Dairy II | lactating cow freestall | August, 07 | – | – | – | – | – | 19 | – | – | – | – |
| | lactating cow exercise yard | August, 07 | – | – | – | 11 | – | – | – | – | – | – |
| | hospital pen | August, 07 | – | 15 | – | – | – | 30 | – | – | – | – |

[a] Units and wells not identified had no detectable concentrations over the course of the study. *: degradation product, –: below detection, NA: not analyzed, NC: not collected. Below detection: Dairy I: Groundwater: well no. 1, soil: lactating cow freestall, lactating cow exercise yard. Dairy II: Groundwater: well nos. 2, 4, 5, 7, 8, soil: heifer yard, calf hutches. Below detection in water samples: Carbamazapine, Azithromycin, Erythromycin, *Erythromycin-$H_2O$, Roxithromycin, Virginiamycin, Ciprofloxacin, Lomefloxacin, Norfloxacin, Ofloxacin, Sarafloxacin, Enrofloxacin, Sulfachloropyridazine, Sulfadiazine, Sulfathiazole, Chlorotetracycline, *Epi-chlorotetracycline, *Epi-iso-chlorotetracycline, *Iso-chlorotetracycline, Doxycyline, *Epi-oxytetracycline, Chloramphenicol, Ormetoprim, Trimethoprim. Below detection in solid samples: Carbamazapine, Roxithromycin, Virginiamycin, Ciprofloxacin, Lomefloxacin, Norfloxacin, Ofloxacin, Sarafloxacin, Enrofloxacin, Sulfachloropyridazine, Sulfadiazine, Sulfathiazole, Doxycyline, Chloramphenicol, Ormetoprim.

**Occurrence and Transport: Animal Production Area.** *Surface and Soil Samples.* The main sources of antibiotics in the animal production area are feces and urine excrements, accumulating in a spatially heterogeneous pattern. Consequently, concentration variability was high despite compositing samples from 12 separate locations across each management unit (Table 3). Sulfonamides (mainly sulfadimethoxine) and tetracyclines were frequently detected in surface samples. High variability was most evident for erythromycin in the lactating cow exercise yard at Dairy I, and for oxytetracycline in the heifer exercise yard and in the calf hutch area at Dairy II: Each was detected at high concentrations (188 to >1000 $\mu$g kg$^{-1}$) once, but was below detection limit at other sampling times. This suggests a very high concentration in one or a few of the samples composited for analysis, which likely resulted from intermittent and spatially variable patterns of administration and excretion. At the two dairies, antibiotics (except monensin at Dairy I) are not administered as feed additives. Only a small number of animals are under treatment at any given time, which results in spatially and temporally variable detections.

Antibiotics were frequently detected in surface samples of hospital pens at both of the dairies. Sulfadimethoxine (5.8−457 $\mu$g kg$^{-1}$) and tetracycline (6.2−73 $\mu$g kg$^{-1}$) were most common. Detections of other antibiotics at hospital pens included oxytetracycline (11 and 18 $\mu$g kg$^{-1}$), and epi-tetracycline (11 $\mu$g kg$^{-1}$).

Detections were sporadic in the surface samples of lactating cow freestalls, lactating cow exercise yard, heifer exercise yard, and calf hutches. Concentrations were similar to those reported elsewhere (*48–50*). We anticipated that high usage of antibiotics in calf hutches would yield numerous detections samples from Dairy II, but obtained few. Limited detections in surface samples at calf hutches at Dairy II were surprising also in light of the frequent and high detections in wastewater samples from calf hutches at Dairy I. We speculate that sulfonamides, which are commonly administered to calves, show low sorption to soils (*26, 27*).

Soil samples (0−30 cm depth) were used to assess infiltration via pore water and to evaluate storage and buffering by soils during infiltration. Soil samples yielded a different pattern of occurrence from that seen in surface samples. At Dairy I, all antibiotics were below detection in samples from lactating cow freestall soils and from lactating cow exercise yard soils, even though sulfamethazine, oxytetracycline, tetracycline, chlortetracycline, and erythromycin A were sporadically detected in surface samples. At Dairy II, sulfamethoxazole was detected in both surface and soil samples from the lactating cow exercise yard; sulfadimethoxine and tetracycline were detected in surface and soil samples from the hospital pen, all at similar concentration levels (11 to 30 $\mu$g kg$^{-1}$, Table 3). Tetracycline was detected in soil of the lactating cow freestalls at Dairy II, but not in their surface samples. This is likely due to intermittent administration and spatial variability. There were no detections in heifer exercise yard soil and calf hutch soils at Dairy II. Overall, the detection of several antibiotics in soil samples indicates differential mobility of antibiotics in the subsurface environment. Hence, the production area of dairies—even outside the lagoon—cannot be ruled out as a potential source of antibiotics in groundwater.

*Production Area Groundwater.* Shallow groundwater was sampled from wells associated with animal production areas to assess the migration of antibiotics into groundwater. Sulfamethazine was found in well no. 1 (Dairy II) for all sampling campaigns, ranging from 0.088 to 0.14 $\mu$g kg$^{-1}$. Well no. 1 is near freestalls, near the feed and manure solids storage areas, and near possibly leaking, buried flush water pipelines. Tylosin and sulfadimethoxine were also detected in ground-

TABLE 4. Approximate Mass of Antibiotics [g] within Lagoons, Groundwater, Management Unit Surface, and Within the 0−30 cm Soil Horizon Calculated by Multiplying the Average Concentration with the Lagoon Volume, Groundwater Volume in the Monitoring Well Source Area and Areas of Each Management Unit, Respectively[a]

| | Dairy I | | | | | Dairy II | | | |
|---|---|---|---|---|---|---|---|---|---|
| | lagoon | lagoon sediments | ground water | surface | soil | lagoon | ground water | surface | soil |
| tylosin | – | – | – | – | – | – | 0.01 | – | – |
| sulfadimethoxine | 402 | – | 0.1 | 34 | – | 36 | 0.01 | 1 | 7 |
| sulfamethazine | 550 | 316 | 4.1 | 6 | – | 14 | 0.3 | 9 | 170 |
| sulfamethoxazole | 103 | – | – | 4 | – | – | – | 31 | – |
| total chlorotetracycline | – | 1543 | – | 1 | – | – | – | – | – |
| iso-chlorotetracycline | 42 | – | – | – | – | 3 | – | – | – |
| epi-iso-chlortetracycline | 29 | – | – | – | – | 2 | – | – | – |
| oxytetracycline | 18 | 956 | – | 30 | – | 11 | – | 180 | – |
| tetracycline | 2 | 368 | – | 76 | – | 6 | – | 1 | 55 |
| epi-tetracycline | 6 | – | – | 98 | – | 1 | – | 0.3 | – |
| lincomycin | – | – | 0.2 | – | – | 3 | 2.2 | – | – |
| trimethoprim | 0.4 | – | – | – | – | – | – | – | – |
| total erythromycin A | – | – | – | 767 | – | – | – | – | – |

[a] Assumptions are well source area 15 m wide by 100 m long and affecting an average depth below the water table of 3.5 m with aquifer porosity of 30%, surface depth 5 cm, soil depth 30 cm, the soil density 1.8 g $cm^{-3}$, the lagoon sediment depth 0.8m, the lagoon sediment density 1.0 g $cm^{-3}$, and lagoon sediment moisture content 40%. Lagoon sediments at Dairy II were not collected.

water below animal production areas, but the detections were sporadic.

**Comparing Shallow Groundwater Impact.** Our study indicates that antibiotics occur ubiquitously at the surface and in the waste-stream of dairy farms, but do not extensively accumulate in soils. They are not generally transported in groundwater beyond the boundaries of the farm—even after decades of use. Sulfonamides, tetracyclines, and their epimers/isomers, and lincomycin were most commonly detected. Tetracyclines and sulfonamides yielded contrasting patterns of occurrence in soils due to their different physicochemical properties. Lincomycin persisted in groundwater, but was not detected in surface or soil samples. Sorption of lincomycin to clay by cation exchange can potentially be significant, but may be inhibited due to high pH, lack of clay minerals with high cation exchange capacity and/or surface area, or the presence of competing cations (*51*).

Based on measured average antibiotic concentrations, total quantities of antibiotics present at the study farms can be computed (Table 4). The known antibiotics mass in groundwater is small compared to other environmental compartments, partly due to the limited extend of the source area associated with the monitoring wells. Tetracyclines exist mainly in lagoon sediments and surface samples while sulfonamides are dominant in lagoon water. The mass of sulfamethazine is also significant in lagoon sediments.

Importantly, sulfamethazine concentrations in the animal production area groundwater were an order of magnitude lower, and those in groundwater from manure-treated fields were 2 orders of magnitude lower than in the lagoon seepage plume (lagoon wells). Furthermore, the concentration in field wells decreased with distance from the flood irrigation system outlet to below detection, similar in occurrence to monensin (*15*). A considerable loss of sulfonamides in soil pore water and in leachate was observed elsewhere with concentration distributions indicating preferential flow (*39, 52*).

These differences in shallow groundwater antibiotics concentrations are partly attributable to differences in loading rates: Lagoons continuously supply antibiotics-containing water to the lagoon plume, while the animal production area receives intermittent, spatially heterogeneous loading, albeit at possibly high concentrations. Manure and lagoon-water application to fields are infrequent and diluted with irrigation water. Based on known hydrologic fluxes and nutrient management practices (*17*), we estimate that the annual average net application of liquid manure in fields is 5 times lower than the potential leaching rate from lagoons. This is consistent with a nearly 2-fold difference in groundwater salinity, a conservative measure of the manure-derived fraction of groundwater (*16*).

After accounting for dilution, biochemical sulfonamide attenuation below fields is between 1 and 2 orders of magnitude larger than below the lagoon or below the production areas. Differences in oxygen content and redox conditions along the flowpaths, and in the concentrations of sulfonamides (*53*) may explain the contrast in biodegradation between these sites: An anaerobic zone exists below the lagoon and extends for at least a few tens of meters (laterally) into the shallow groundwater (*16, 54*), whereas irrigation water mixed with lagoon water during flood irrigation is sufficiently high in dissolved oxygen to permit aerobic degradation in the subsurface of the field well source area. Production area groundwater also has low redox potential with very low oxygen content. This is consistent with previous work indicating that the major attenuation process of sulfonamides is aerobic biodegradation, but not complete mineralization to $CO_2$, and sorption of the degradation products to soil (*52*). In addition, Wang et al. showed that sulfadimethoxine biodegradation is faster when the initial concentrations are lower (low mg $kg^{-1}$ range) (*53*). However, it is not clear if this qualitative relationship between biodegradation and initial concentration can be extrapolated to liquid concentrations at the ng $L^{-1}$ level observed here.

Our results suggest that sulfonamide attenuation can be improved by proper dilution of lagoon water with irrigation water and control of the loading rate. This will provide sufficient labile organic matter to stimulate microbial activity, while avoiding pervasive anaerobic conditions. Longer flowpaths to promote sorption may further facilitate concentration reduction in groundwater. Future research is needed to identify attenuation mechanisms that can be tied to specific best management practices (BMP) including dilution ratio and irrigation practices to optimally promote degradation and sorption.

Further research must assess whether the low but continuous occurrence of antibiotics at the farm surface affects the ecosystem and microbial community including development of antibiotic resistance. Localized high concentrations of antibiotics at dairy facility surfaces also suggest that the atmospheric pathway via dust emissions deserves close attention. Degradation pathways and physicochemical and degradation properties of parent and degradation compounds urgently need further study and aggregation into a publicly accessible database.

Importantly, our work shows that the distinction of management units by antibiotic use patterns and by operational system is important to understanding the occurrence of these compounds in animal farming operations. The large spatial and temporal variability suggests that intensive sampling campaigns are necessary to properly evaluate animal farms as sources of antibiotics.


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## Supporting Information Available

Analytical methods, study-site farm layouts, theoretical worst-case maximum lagoon concentration estimate.This material is available free of charge via the Internet at http://pubs.acs.org.

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