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Research Report

A REVIEW OF THE LITERATURE ON THE OCCURRENCE AND SURVIVAL OF PATHOGENS OF ANIMALS AND HUMANS IN GREEN COMPOST

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THE OCCURRENCE AND SURVIVAL OF PATHOGENS OF ANIMALS AND HUMANS IN GREEN COMPOST

Contents

Summary

A large number of pathogenic viruses, bacteria, protozoa and parasites may gain access to waste materials including those destined for composting. It is possible to compile extensive lists of organisms which may contaminate wastes and present a risk of human or animal infection when they are utilised. For most the risk is theoretical. It has been common practice for thousands of years to dispose of human and animal excreta on land. Problems presented by such disposal have recently been exacerbated by the intensification of agriculture, the growth of the human and farm animal population and the popularity of organic farming methods. The agricultural or domestic use of compost may possibly increase the risk of disease transmission by direct contact of humans or animals with the material or by contamination of food crops or by adding to environmental contamination which may maintain diseases in the food animal population. It is, however, doubtful whether the use of compost will create any greater hazard than the use of human and animal wastes as agricultural fertilisers.

In the UK the diseases of humans which should be considered most important are probably foodborne infections and intoxications caused by *Salmonella*, pathogenic *E. coli* (particularly enterohaemorrhagic *E. coli* O157:H7), *Campylobacter*, *Listeria*, *Yersinia*, *Shigella*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium botulinum*, *Staphylococcus aureus*, hepatitis viruses, Norwalk virus, Arboviruses and Caliciviruses. Pathogens of animals should also be considered. These include zoonotic agents such as *Salmonella* and *E. coli,* but also endemic agents such as *Mycobacterium bovis*, *Mycobacterium paratuberculosis* (Johne's Disease) Bovine Viral Diarrhoea Virus (Mucosal Disease) and *Serpulina hyodysenteriae* (Swine Dysentery) and exotic agents including Foot and Mouth Disease Virus, Classical Swine Fever Virus and Aujesky's Disease Virus which can be transmitted by food wastes.

Most pathogens are inactivated by the composting process and a composting procedure with a residence time of 3 days at a temperature greater than 55°C results in a sanitised compost. Resistant organisms such as *C. perfringens*, *C. botulinum* and the cysts and eggs of protozoan and helminth parasites may survive. There is also a danger that *E. coli* and *Salmonella* may grow in the final compost if the process has been inefficient and the organic matter remains poorly stabilised. Other bacteria such as *Campylobacter* will only grow within an animal host and viruses, most protozoa and parasites require animal tissue in which to replicate.

Future research should concentrate on determining the conditions for the survival of *E. coli*, *Salmonella* and heat-resistant bacteria such as *C. perfringens* in experimental and industrial composting systems more precisely. The possible role of compost in the spread of *C. botulinum* should be investigated although the risk from this organism is probably theoretical. There is a shortage of information on the survival of viruses during composting and particularly exotic viruses such as Foot and Mouth Disease Virus and Classical Swine Fever Virus.

Introduction

The purpose of this short report is to review recent scientific literature relating to the survival of microbial pathogens of animals and humans during composting, with particular emphasis on green waste composts. The report would be used to inform a decision of pathogens which may be used experimentally to evaluate composting processes for green wastes. The volume of literature, and particularly recent publications, on the survival of pathogens during composting is small and therefore information has also been drawn from investigations on the occurrence and survival of pathogens in animal wastes, particularly slurry, and human sewage sludge.

It has been estimated that several hundred diseases may be transmitted from animal to animal and that more than one hundred and fifty may be transmitted from animal to man (Diesch, 1974; Strauch, 1978; Strauch, 1994). The causative organisms of such diseases are often excreted in the faeces, urine and other fomites of diseased individuals, recovered and convalescent cases and healthy "carriers", even when the disease is systemic rather than enteric.

The spread of many human diseases such as cholera, typhoid and dysentery has been controlled by isolating man from human excreta and improvements in personal hygiene and the use of sewage and water treatment processes. In areas of the world where such improvements have not been made, or systems are inefficient, such diseases remain endemic. Although improvements in animal husbandry systems have been made this isolation has not been possible for the farm animal population.

Before the intensification of agriculture, and the increase in the size of herds and flocks, a simple separation of farm animals from their excreta was achieved by collecting faeces and urine and removing it to a midden to compost. The resultant compost was thought to be free of most pathogens and safe when spread on pasture or crops (Thunegard, 1975).

It has been common practice for thousands of years to dispose of human excreta on land. Problems presented by such disposal have been exacerbated recently by the intensification of agriculture, the growth of the human and farm animal population, the cost of artificial fertilisers, which renders materials such as sewage sludge, previously regarded as waste, a valuable commodity, and the growth of organic farming methods.

Both animal and human wastes may be used in agriculture following composting. Composting may also be applied to other waste products to release nutrients and render them safe to use. This includes green compost composed of waste plant material but which may also contain animal waste, human sewage and catering waste and which may be contaminated with the excreta of farm animals, wild animals and pets such as dogs.

If diseases are to be spread by wastes and processed wastes the material must become infected with the causative organisms, which must survive treatment or storage, remain capable of causing disease and survive in the material until a human or animal host is encountered. Since a large variety of pathogenic organisms may be excreted by infected animals it may be assumed that a similar number of organisms may be found in wastes or compost at some time. In addition materials such as green compost may become contaminated by organisms such as *Clostridium perfringens* which are common inhabitants of soil but which may occasionally cause disease in humans or animals.

The agricultural or domestic use of compost may possibly increase the risk of disease transmission by a number of mechanisms. The most obvious is direct contact by humans handling the material or animals grazing pastures or crops on which the material has been used as a fertiliser. This could be particularly important for organisms such as entrohaemorrhagic *Escherichia coli* O157:H7 which is thought to have a low infectious dose (possibly as few as 10 cells may initiate an infection in susceptible humans; Tarr, 1995; Anon, 1997). Indeed, direct contact with ruminant faeces on farms and on contaminated pastures has resulted in outbreaks of *E. coli* O157:H7 (O'Brien *et al.*, 2001; Ogden *et al.*, 2002. For a review see Stevens *et al.*, 2002). Disease, and particularly enteric disease, may also occur following contamination of food

crops. Outbreaks of *E. coli* O157:H7 have also resulted from the consumption of water, unpasteurised apple drinks and vegetables contaminated with ruminant faeces (Olsen *et al.*, 2002; Cody *et al.*, 1999; Hillborn *et al.*, 1999). The use of compost could also provide a mechanism for the maintenance of organisms such as *Salmonella*, *E. coli* and *Campylobacter* in the environment. The main source of these organisms for the human population is the consumption of contaminated animal products. The environment is, however, already heavily contaminated with a variety of disease organisms, some of which, such as *C. perfringens* and *Listeria*, occur extensively in soil. It is doubtful whether the use of compost will create any greater hazard than the use of human and animal wastes as agricultural fertilisers.

It is possible to compile extensive lists of organisms which may contaminate wastes (Ellis and McCalla, 1978) and present a theoretical risk of human or animal infection when they are utilised. In practice, however, most organisms will be killed by treatment, particularly composting (Platz, 1977) or will be present in numbers which are unlikely to cause disease. Organisms which may contaminate green compost in the UK include bacteria such as *Salmonella*, *Campylobacter*, *E. coli* (including enteropathogenic and enterohaemorrhagic types such as O157:H7), *Pasteurella*, *Listeria*, *Erysipelothrix*, *Staphylococcus aureus*, *Leptospira*, *Serpulina hyodysenteriae*, mycobacteria including perhaps *M. bovis* and *M. paratuberculosis* (Johne's disease), spore-formers such as *C. perfringens* and *C. tetani* and rickettsias such as *Coxiella burnettii* (Q fever). The majority of them are thought not to survive composting of animal manures although some, such as *Listeria*, mycobacteria and spore-formers, may be more resistant. The latter are, however, common contaminants in soil and their presence in compost would probably not create an increased hazard.

Most pathogenic bacteria, including salmonellas and *E. coli* are adapted to grow in the tissues or fluids of their host at a temperature of approximately 37^{o}C and when released from this environment their numbers usually decline exponentially. Thus, although multiplication of salmonellas in slurries and sludges and in water contaminated with faeces has been reported and although nutrients necessary for growth are available it is generally accepted that they do not increase outside their host (Jones, 1986). The growth of *E. coli* in sanitised compost when sufficient nutrients are available has been reported (Soares *et al.*, 1995). Russ and Yanko (1981) found that *Salmonella* sp could grow in composted sewage sludge if the carbon/nitrogen ratio was greater than 15 and the manure content greater than 20*per cent*. The regrowth rate of *Salmonella* in stabilised wastes may be associated with the indigenous microflora (Hussong *et al.*, 1985). It has been suggested that the active indigenous flora of compost establishes an homeostatic barrier to colonisation by *Salmonella* and in its absence re-inoculated *Salmonella* my grow to a potentially hazardous level. However, *Salmonella* are not usually found in commercial composts and probably only grow in materials which have been sterilised (Brandon *et al.*, 1977) and Russ and Yanko (1981) concluded that the native microflora inhibited the growth of salmonellas in composted sludges. This highlighted the fact that when composting processes are carried out in an inefficient manner, the organic matter remains poorly stabilised and sanitised, recontamination can occur and the compost may become a source of pathogens. This is only a problem with bacteria such as *E. coli* and *Salmonella* which can occasionally grow outside their host. Other bacteria such as *Campylobacter* will only grow within an animal host and viruses, most protozoa and parasites require animal tissue in which to replicate.

Occurrence of pathogens in human and animal waste and compost

The occurrence and survival of pathogens in human sewage and animal wastes and the subsequent survival and infectivity of organisms when these materials are disposed of on land has been extensively reviewed (Strauch *et al.*, 1978; Dumontet *et al.*, 1999; Ellis and McCalla, 1978; Jones, 1980; Jones, 1981; Jones, 1981; Jones, 1982; Jones, 1984; Jones, 1986; Jones, 1992; Jones *et al.*, 1980; Strauch, 1996; Strauch and Ballineri, 1994) (Table 1). Jones (1982) described the pathogens most commonly found in animal wastes (Table 2) which could be transmitted to other animals. This list could be extensively updated, but the main risk organisms are still *Salmonella*, pathogenic *E. coli*, *M. tuberculosis* and *M. bovis*. In agricultural use of animal wastes these organisms can be controlled by codes of practice limiting spreading and grazing by animals (Jones, 1982). Pell (1997) indicated that there are numerous pathogens in livestock manure which can infect humans. The principal ones are *Crytosporidium parvum*, *Giardia* sp., *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* sp. and *M. paratuberculosis*. The type of pathogens most commonly found in sewage

and sewage sludge depends on the state of health of the population, as well as the presence of hospitals, meat processing plants and abbatoirs in the area (Bruce and Davis, 1983; Jones *et al.*, 1980a; Jones *et al.*, 1980b). Organisms commonly encountered include *Salmonella* and *E. coli*, but a number of others including streptococci, *Campylobacter* and mycobacteria may be present usually at a lower concentration. In an extensive review Epstein (2002) reported on the occurrence of pathogens in biosolids, municipal and waste solids, food waste, wood waste and yard waste. Organisms reported included bacteria such as *Salmonella*, *Shigella*, *Yersinia*, *M. tuberculosis*, *Vibrio cholerae*, *E. coli*, *Listeria* and a number of viruses, protozoa and parasites. Many are rare or do not occur in the UK (Tables 3-7).

In the UK the diseases of humans which are most important are probably foodborne because there is a potential for compost to increase the risk of infection. These are *Salmonella*, *E. coli* (and particularly enteropathogenic [EPEC], enterohaemorrhagic [EHEC] and enterotoxigenic [ETEC] strains), *Campylobacter jejuni* and *C. coli*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Shigella dysenteriae*, (and others, including *S. sonnei*), *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Clostridium botulinum*, hepatitis viruses, Norwalk virus, Arboviruses and Caliciviruses (Cook, 1991). Of these, the most important are *Salmonella* and *Campylobacter* since infections with these agents are predominant, and enterohaemorrhagic *E. coli*, particularly *E. coli* O157:H7 because infections, although far less common than *Salmonella* and *Campylobacter*, are life-threatening in susceptible populations. *Salmonella* is endemic in poultry, cattle, pigs and sheep and pets in the UK. The organism has declined in humans and poultry but its prevalence in cattle and pigs is probably increasing. There is a great potential for the contamination of green compost if it contains faeces from farm animals, humans or pets.
Infections by *Campylobacter* are the most frequent cause of enteritis in humans (Koenrad *et al.*, 1997; Tauxe, 1992). Undercooked poultry products, raw milk, untreated surface water and pets are probably the most important vectors but the organism is almost universally present in farm animals and particularly poultry. Fortunately, and unlike *Salmonella* and *E. coli*, this organism does not grow outside a mammalian or avian host and this may reduce the risk of disease transmission *via* compost, although it may play a role in maintenance of the organism in the environment.
Enterohaemorrhagic *E. coli* O157:H7 occurs predominantly in ruminants (Stevens *et al.*, 2002) but also in wild animals, birds and domestic pets (Mainil, 1999). Wild and domestic animals may act as vectors of transmission of EHEC to farm animal hosts and, on rare occasions, humans. Indeed, wild rabbits were implicated in an outbreak of EHEC infection in visitors to a wildlife centre in England (Pritchard *et al.*, 2001), probably following contamination of picnic areas with rabbit faeces.

Healthy cattle and sheep sporadically carry *E. coli* O157:H7 in their gastrointestinal tract and shed the bacteria in their faeces (Chapman *et al.*, 1993; Hancock *et al.*, 194; Kudva *et al.*, 1997; Kudva *et al.*, 1996). In cattle, *E. coli* O157:H7 occurs with an overall prevalence of 0.3 to 6.1*per cent* and the average time that faeces from an individual animal remains positive is 30 days (Sanderson *et al.*, 1995; Wells *et al.*, 1991; Kudva *et al.*, 1996; Paiba *et al.*, 2002). In sheep the prevalence is 0.9 to 31*per cent* and the organism is shed in faeces for more than a year (Kudva *et al.*, 1997; Kudva *et al.*, 1996; Zhao *et al.*, 1995). Contamination of non-ruminant food sources of infection is most often from ruminant manure (Samadpour *et al.*, 1994; Tarr, 1995; Waters *et al.*, 1994) and vegetables associated with outbreaks were found to have been grown in soil layered with manure (USDA, 1997; Cieslak *et al.*, 1993).

Boulter *et al.* (2002) carried out an extensive study of bacteria isolated from green compost. The majority of organisms were Gram-negative pathogens, or potential pathogens including *Salmonella*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Bordetella bronchiseptica*. The latter is a specific pathogen of farm animals and only the organisms previously listed would indicate a possible risk to humans or animals exposed to the end product.

Pathogens of farm animals should also be considered. These include human pathogens such as *Salmonella* and *E. coli* but should also include diseases of cattle such as bovine tuberculosis, Johne's Disease (*M. paratuberculosis*), Mucosal Disease, (Bovine Viral Diarrhoea Virus) and disease of pigs such as Swine Dysentery (*Serpulina hyodysenteriae*). Of particular importance are OIE List A diseases which do not normally occur in the UK including Foot and Mouth Disease, Classical Swine Fever and Newcastle Disease. These and Aujesky's Disease (pseudorabies) can be transmitted via food wastes (Strauch, 1996) and are an obvious hazard. Table 1 lists organisms thought to present a disease risk (Strauch, 1996).

Survival of pathogens in human and animal waste and compost

Introduction

Boulter *et al.* (2002) defined composting as "intense microbial activity leading to decomposition of most biodegradable materials (Weltzein, 1991; Adani *et al.*, 1997). This biological process involves the complete or partial degradation of a variety of chemical compounds by a consortium of micro-organisms, the composition of which changes as composting progresses (Whitney and Lynch, 1996). During composting, the microbiological community follows a predictable successional pattern." Microbial activity is a prerequisite for a satisfactory composting process (Befta *et al.*, 1996). Mesophilic, thermotolerant and thermophilic bacteria, actinomycetes and fungi are involved in the composting process. Similarly, the physical and chemical conditions involved during composting are variable and these may alter the results of the process unpredictably (Richard and Zimmerman, 1995). It is, therefore, not surprising that reports of the survival of microbial pathogens in composted wastes are variable.

Typically, temperatures reached in a well-managed compost operation are in a range of 50 to 65°C. Such temperatures are well above the thermal death points of mesophilic pathogens (Golueke, 1982). As the temperature of the composting process increases pathogens are usually destroyed as they reach their thermal death points (Table 8). The survival of bacteria is variable but most viruses are killed in about 20 minutes at 70°C (Day and Shaw, 2000). There is a relationship between temperature and time. A high temperature for a short period or a lower temperature for a longer period may be equally effective. Epstein (2001) reported that high temperatures were extremely effective in the destruction of pathogens. The time/temperature relationship for a number of pathogens is shown in Tables 9 and 10. The D-value is the length of time required to obtain a ten-fold reduction in the number of organisms.

The absolute time to extinction of an organism is less important than the rate at which the concentration declines. Although the former is a function of the latter the reporting of the survival time without specifying the rate of decay and decay conditions and how they may affect the rate of decline has resulted in incorrect conclusions. Obviously, the greater the number of pathogens present the longer will be their time to extinction. Unfortunately, many of the studies described below report only the time to extinction, without calculating the rate of decay or describing the conditions.

Farm animal wastes and human sewage sludge

Jones (1982) reviewed information on the survival of salmonellas in cattle slurry. The type of slurry, storage temperature and serotype of *Salmonella* all affected the survival time which also depended to a considerable extent on the concentration of *Salmonella* at the beginning of storage – the higher the concentration of organisms at the commencement of storage the longer the time to extinction. Survival was enhanced by reduction in temperature and an increase in dry matter. Survival was greatest at temperatures below 10°C and in slurries containing more than 5 *per cent* solids. During storage the pH of slurry drops from an average value of 7.5 to below 6.5 during the first month, before returning to approximately 7.0 (Jones, 1976). A few salmonellas may survive for in excess of 150 days, but 90 *per cent* die during the first 2 to 4 weeks, while the pH is falling. This decline in pH during storage is related to the production of fatty acids by the natural bacterial flora. Their production combined with the inability of salmonellas to compete for nutrients with the natural bacterial flora of slurry probably accounts for their death (Jones, 1976).

The reduction of pathogens in sewage sludge by composting has been extensively reviewed by Dumontet *et al.* (1999). Sludge may contain a large variety of bacterial and viral pathogens including *Salmonella*, *Shigella*, *Yersinia*, and enteroviruses as well as eggs of parasites such as *Ascaris lumbricoides*, *Cryptosporidium* and *Giardia*. Competition and depletion of nutrients combined with increased temperature results in the inactivation and destruction of pathogens (Stentiford, 1986). A composting procedure with a residence time of 3 days at a temperature of greater than 55°C resulted in a sanitised compost.

Indicator organisms and pathogenic E. coli and Salmonella

Pereiro Neta *et al.* (1986) studied the survival of indicator organisms in sewage sludge composted with solid municipal wastes. The organisms used to monitor the effectiveness of the composting process to produce a

sanitised waste were *E. coli*, faecal streptococci and *Salmonella*. They concluded that static aerated piles were more efficient than windrows in the inactivation of the indicator organisms. In static aerated piles *E. coli* was reduced below the detection level, faecal streptococci were reduced to less than 10^2 cfu per gram and *Salmonella* were completely eliminated after 32 days of composting. In contrast, all of these organisms were still detectable at the end of the windrow composting process. Hayes (1996), however, reported that in a well-managed biosolids windrow composting operation indicator organisms and pathogens including *E. coli* and *Salmonella* were destroyed and Gaby (1975) found that *Salmonella* and *Shigella* disappeared in windrow composting of municipal solid wastes in 7 to 21 days

The survival of *E. coli* O157:H7 in green compost has not been reported. Maule (1998) reported that *E. coli* O157:H7 survived for more than 56 days in fresh cattle faeces and up to 9 days in cattle slurry at 18 °C, while Kudva *et al.* (1998) were able to isolate this organism from a manure stack for as long as 21 months. Similarly, *E. coli* O157:H7 appears to survive for long periods in bovine manure mixed with bedding. Wang *et al.*, 1997, reported the survival of an initial inoculum of 10^5 cfu per gram of this organism for 70, 56 and 49 days respectively at 5, 22 and 37°C. Kudva *et al.* (1998) demonstrated that *E. coli* O157:H7 survived in a manure pile from sheep for 21 months. In a pile which was aerated the organism survived for 4 months. In bovine manure the survival time was 47 days. Once the manures were aerated and heated survival times were reduced such that at temperatures above 45°C the organism was not detected after 2 days. Himathongkham *et al.* (1999) reported that this organism declined exponentially in cattle manure with a decimal reduction time (DRT) of from 6 days to 3 weeks and in cattle slurry with a DRT of 2 days to 5 weeks. The most rapid destruction was achieved at 37°C and numbers of *E. coli* declined at approximately the same rate as *S. typhimurium*. In poultry manure the concentration of *E. coli* O157:H7 and *S. typhimurium* also declined exponentially. The DRT ranged from 12 hours to 1-2 weeks at 4°C (Himathongkham *et al.*, 2000).

Survival for shorter periods in composted wastes should be predicted, although Droffner and Brinton (1995) reported the survival of *Salmonella* and *E. coli* in industrial composts for up to 59 days at 60°C. This study relied upon the use of gene probes rather than isolation of viable organisms, which may account for the extended survival period reported. In contrast, Turner (2002) found that an indicator strain of *E. coli* was inactivated in farmyard manure, pig faeces and straw if kept at 55°C for more than two hours. It was, however, still viable after 72 hours at 50°C. Coliforms grew in the compost if the process was carried out at mesophilic (37°C) temperatures. Knoll (1961) described several experiments where *Salmonella* strains were subjected to different composting temperatures. After 14 days at 55 to 60°C the final compost did not contain salmonellas. Thermal destruction of bacteria may depend on factors other than temperature, including moisture content, ammonia concentration and the presence of other organisms. In industrial compost *Salmonella* and *E. coli* were found to survive for 59 days at 60°C (Doffner and Brinton, 1995). Lung *et al.* (2001) inoculated *E. coli* O157:H7 and *Salmonella enteritidis* into a bench-scale cow manure composting system at a level of 10^7 organisms per gram of raw compost. *E. coli* was not detected after 48 hours at 45°C and *S. enteritidis* after 72 hours. When the compost was held at 25°C the concentration of seeded organisms did not decline. The effect of temperature was also demonstrated by Gibbs *et al.* (1998). They investigated the effect of two windrow composting systems on the survival of *Salmonella*. *Salmonella* was not detected after composting at 52 to 53°C but was isolated from 7 of 11 samples at 30 to 40°C. Tiquia *et al.* (1998) found that *Salmonella* was no longer detected after composting for 3 weeks in a mixture of partially decomposted pig manure and sawdust at temperatures between 64 and 67°C. Hirn *et al.* (1983), using compost derived from sewage sludge and food waste, did not isolate *Salmonella*. Faecal streptococci and *C. perfringens* were always present, although numbers declined by 2 to 3 logs during a 6- to 7-week period. Plym-Forshell (1983) studied the survival of salmonellas in cattle and pig manure mixed with straw. *S. dublin*, *S. senftenberg* and *S. typhimurium* survived for less than 7 days in cattle manure at 55 to 60°C or pig manure at 54 to 60°C. In contrast, *S. derby* persisted for more than 140 days at 2 to 19°C. Russ and Yanko (1981) studied the kinetics of development of sludge compost inoculated with *Salmonella* sp under aerobic and anaerobic conditions. During the anaerobic incubation *Salmonella* sp were detected until the 32nd day of incubation, while during the aerobic incubation *Salmonella* sp decreased to an undetectable level in about 15 days. Burge *et al.* (1978) reported that *Salmonella* were destroyed in 10 days by aerated, static pile composting of raw sludge in 15 days in turned windrows. Similarly, Knoll (1961) described several experiments in which *Salmonella* strains were subjected to composting at a biosolids refuse plant. The salmonellas were eliminated after 14 days at 55 to 60°C. At a higher temperature of 60 to 70°C *S. newport*

could not be isolated from composted sewage after 3 days (Wiley and Westerberg, 1969). At a similar temperature (60 to 65°C) *S. typhimurium* and *Serratia marcescens* inoculated into a composting drum containing septic waste, biosolids and municipal solid wastes could also not be isolated after 3 days (Krogstad and Gudding, 1975).
In an extensive recent study Christensen *et al.* (2002) investigated the survival of indicator organisms (*E. coli* and *Enterococcus faecalis*) and *Salmonella* during composting in 4 facilities in Denmark, Sweden, Norway and Finland. In Denmark, sewage sludge mixed with straw yard waste and straw was composted in open-air windrows without cover or forced aeration for 7 weeks followed by stabilisation for 2-3 months. In Sweden, source-separated household waste mixed with yard waste was composted in force-aerated boxes with semi-permeable covers; after a sanitation phase of 3 weeks the compost was moved to a force-aerated box without a roof for a 4-week stabilisation phase followed by a second stabilisation phase in open-air windrows for an additional 4 months. In Norway, source-separated household waste was mixed with yard and forestry waste and composted in open-air windrows without cover or forced aeration; windrows were combined after 6 weeks and left to stabilise for 6 weeks then 2 to 3 months. In Finland, sewage sludge mixed with wood chips and peat was composted in fully-enclosed, aerated tunnels for 7 days, followed by a second sanitisation period of 7 days in a second tunnel and a stabilisation phase of 6 months in an open-air windrow. During composting variations in temperature at different levels in the compost occurred at all 4 facilities. Mean temperatures were, respectively, 50-66°C, 45-74°C, 62-66°C and 43-57°C, with maxima of 75°C, 74°C, 79°C and 75°C.
Salmonella was present in the raw material at all 4 facilities but, with the exception of the plant in Sweden where 2 of 5 samples of sanitised compost were positive, it was not present in sanitised or finished compost. *E. coli* concentrations were reduced in all facilities and numbers above the detection limit were only observed in 4 of 47 samples analysed. Since concentrations below the detection limit were observed for most of the samples the exact reduction could not be calculated. However, in general, reductions of 4.9 to 6.6 log units were achieved.
The reduction of *Enterococcus* ranged from 4.1 to 5.7 log units. Reduction was less efficient in windrow units. At the facility in Sweden the temperature was significantly lower at the base of the windrow and this coincided with a less efficient reduction of *E. coli* and *Enterococcus*, thus emphasising that temperature is an important factor in the inactivation of these organisms.

Golueke (1982) reported on the survival of a number of organisms during composting (Table 11).

Spore-forming bacteria

A number of bacteria, particularly from the genera *Bacillus* and *Clostridium* produce resistant endospores which enable them to survive for long periods in the environment. Investigations have shown that the temperature resistance of *C. perfringens* results in only small reductions of this organism during composting (Hirn *et al.*, 1983; Molland, 1980). Surprisingly, however, in a composting drum containing septic waste, biosolids and municipal wastes, although *Bacillus cereus* was still detected after 7 days at 60 to 65°C it was not isolated once the temperature reached 70°C (Krogstadt and Gudding, 1975).

Clostridium botulinum occurs extensively in the environment. It elaborates toxins which may cause serious, life-threatening intoxications if ingested in food by humans and animals. It has been suggested (Popoff and Argente, 1996) that the spreading of bio-waste on agricultural land may raise the concentration of *C. botulinum* in the environment and therefore increase the risk to human and animal health. Mitscherlich and Marth (1984) reported that clostridial spores survive a temperature of 100°C at pH6.0 to 7.4 for more than 110 mins, even though the vegetative cell is no more resistant than those of any other bacteria. It may, therefore, be expected that *C. botulinum*, like *C. perfringens*, may survive composting conditions which normally kill other bacteria. Bohnel and Lube (2000) found that 54*per cent* of samples of commercially-marketed bio-compost contained *C. botulinum* and that stabilised compost stored at ambient temperature for 5 weeks to a year still contained the organism. This is obviously important if the organism is allowed to gain access to food in which it may grow and elaborate toxins. Toxin production may also be possible during the composting process (Dezfulian, 1999). This risk is, however, similar for human and animal wastes applied to agricultural land and, since the organism is already present in soil, it remains to be determined whether bio-compost presents an increased risk.

Other bacteria

There is little information on the survival of other bacteria during composting. Gaby (1975) reported a shorter survival time for *Leptospira philadelphia* than enteric organisms. The former survived for only 2 days in windrow-composted municipal waste solids, compared to 7 to 21 days for *Salmonella* and *Shigella*. *Mycobacterium tuberculosis* was killed in a similar system by 14 days at 65°C (Morgan and McDonald; 1969).

Viruses

Although viruses of human and animal origin have been found in wastes (Turner *et al.*, 1999) including refuse (Golueke, 1977) their survival during waste treatment has not been well-studied.

In trials in which cattle manure was inoculated with a bovine enterovirus and a bovine parvovirus (Monteith *et al.*, 1986) both were inactivated within 30 minutes of thermophilic anaerobic digestion at 55°C and neither virus survived aerobic composting for 28 days at 60°C. Similarly, Turner *et al.* (1999) reported that Swine Vesicular Disease Virus in pig slurry in an experimental pilot plant was inactivated after 5 minutes at 50 to 55°C at alkaline pH (7.5 to 8.0) and at 55 to 60°C at pH 6.4. African Swine Fever Virus was inactivated at 50°C at pH 8.

Information is available on the destruction of poliovirus by composting. Wiley and Westerberg (1969) reported that type 1 poliovirus was killed by composting of human sewage at 60 to 70°C for 3 days, whilst Gaby (1975) found that type 2 virus could not be detected after composting of municipal solids wastes for 3 to 7 days. Inactivation is probably related to temperature. Ward and Brandon (1978) demonstrated that Poliovirus was progressively inactivated at temperatures between 35 and 47°C in composted biosolids. At 47°C inactivation occurred after 5 minutes.

Because of their increased resistance it has been suggested that bacteriophage may be used as indicators of composting efficiency. Compared to other viruses and most bacteria their survival time during composting is extended. Burge *et al.* (1978) reported that t2 bacteriophage survived for 20 days in an aerated static pile and up to 70 days in windrows. This compared to the survival of *Salmonella* for 10 and 15 days respectively in the same systems.

Agents of Transmissible Spongiform Encephalopathies

Some mention should be made of the agents of transmissible spongiform encephalopathies (TSEs: Scrapie, Bovine Spongiform Encephalopathy, Creutzfeldt-Jakob Disease, v-Creutzfeldt-Jakob Disease, Chronic Wasting Disease, etc.) although these agents are not likely to be transmitted or maintained in the environment by compost (Epstein, 1997). TSEs are highly resistant to heat and chemical inactivation and their survival in the environment is probably prolonged. They may persist in soil for several years. They are not thought to be excreted in the faeces or urine of infected individuals although they could gain access to waste if infected animal tissues are not disposed of correctly. In the UK specified risk materials from cattle and sheep (SRM) are usually disposed of by incineration. The epidemic of BSE in the UK which developed in the 1980s is now declining. Scrapie is still endemic in sheep and it is not clear whether BSE has established itself in the sheep population (Baylis *et al.*, 2002). TSE agents are unusual in that the infective particle may be a protein and unlike other "organisms" may not contain nucleic acid (McKinley *et al.*, 1983; Prusiner *et al.*, 1982). Their normal route of infection is by ingestion, direct injection, scarification or maternal transfer (Pattison *et al.*, 1972; Taylor *et al.*, 1996; Dickinson, 1976) but concentrations in compost are unlikely to cause a problem. They do not increase in concentration outside their host. Because of the difficulty in recovering TSE agents their presence in the environment has rarely been studied and there is no information on their survival during composting.

Protozoa and parasites

The nematode *Ascaris lumbricoides* is amongst the most cosmopolitan and most common of all pathogens of humans (Hannan, 1981). They may survive for several years in the environment (Feachem *et al.*, 1983). In their fully-developed, second-stage larval form, eggs of *Ascaris* are highly resistant and are frequently used as indicator organisms for water and sewage treatment processes. They may, therefore, be an ideal

indicator for the effectiveness of composting to reduce parasites (Mara and Cairncross, 1989). Meekings *et al.* (1995 and 1996) studied the survival of *A. galli* eggs, which they used as a model of *A. lumbricoides*, in municipal waste compost and sewage sludge compost at 30°C. They demonstrated that fully-developed eggs could still be observed after 20 days of composting, although these had declined from 60 *per cent* to approximately 12 *per cent* during this period. Hays (1996) reported that *Ascaris* ova were destroyed in a well-managed windrow composting system and While and Westerberg (19690 found that the aerated pile method was also effective in destroying *A. lumbricoides* eggs, which were killed in composted sewage after 3 days at 60 to 70°C.

Other parasites and protozoa which may occur in compost are listed in Table 1 and Table 5, but little information on their survival is available. It may be expected that the survival time of protozoa such as *Cryptosporidium* and *Giardia* may be extended, since both may remain viable in the environment for more than a year (Current, 1998). *Giardia* cysts were still detected after windrow composting at 52 to 53°C although it was not certain whether they were still viable (Gibbs *et al.*, 1988). In contrast, *Entamoeba histolytica* and *Endolimax nana* had disintegrated after windrow composting of municipal solid wastes after 7 days. The parasites *Necator americanus* and *Ancylostoma duodenale* were also killed by the same conditions.

Standards for pathogens in compost

It is not the purpose of this review to discuss standards for composting of wastes or methods of assessing the efficiency of the composting processes. It has been proposed that the attainment and maintenance of a temperature higher than 55°C over a 3-day period should have been sufficient to have eliminated all pathogens (Anon, 1981) and Strauch (1996, Table 12) documented the requirements proposed in a number of countries. A list of organisms which could be used as indicators of the efficiency of processing has been proposed (Anon, 2000, Table 13). Sanitisation standards for compost have been developed in the USA (composting Council of the US, 1993; Leege and Thompson, 1997) and in the UK by the Composting Association (2000). The latter have been reviewed recently in a WRAP-funded project and specify minimum compost temperatures of 55-65°C for periods of 3 to 14 days depending on the composting process (turned windrow, in-vessel, static aerated piles). A risk assessment of composting treatment to dispose of catering waste containing meat recommended a minimum composting temperature of 60°C for 2 days (Gale, 2002). This was based on the eradication data for a large number of animal pathogens. However, there may be considerable differences in composting temperatures between composting systems of the same 'category' depending on dimensions, airflow and ambient temperature (Bertoldi *et al.*, 1996; Stofella and Kahn, 2000). The US EPA in "Processing to Further Reduce Pathogens" (Composting Council, 1993) established criteria for composts made with biosolids. According to the Federal Biosolids Technical Regulations, a windrow must reach a minimum temperature of 55°C for 15 days, with a minimum of 5 turnings. For an in-vessel or static pile system a minimum temperature of 55°C for 3 days is required.

Conclusions and gaps in research

- A large number of pathogenic viruses, bacteria, protozoa and parasites may gain access to waste materials including those destined for composting.
- To cause a hazard they must survive the composting process and be able to gain access to their host, either directly or by contaminating pasture or food crops.
- Composts may also play a role in maintaining the presence of pathogens in the environment where they may be ingested by food animals. This risk is probably less than that presented by the use of human sewage, sewage sludges and animal wastes in agriculture as fertilisers.
- Most pathogens are efficiently removed during the composting of green waste as long as a temperature of 55°C for 3 days is achieved.
- Although there is an extensive literature on the survival of human and animal pathogens in farm animal and human sewage wastes less information is available on the survival of pathogens during green waste composting.
- Several hundred pathogens could be disseminated to animals and humans by the use of green waste composts. In practice the risk is probably low and can be restricted to a few organisms which have a low infectious dose or may 'regrow' in the finished product. This may include salmonellas and enterohaemorrhagic *E. coli*.
- Animal and human wastes have been used in agriculture as fertilisers and as a method of disposal for thousands of years. Infections in humans and animals because of this practice have only seldom been recorded.
- The most important agents for humans are those which cause food-borne infections including *Salmonella*, *E. coli* O157:H7 and *Campylobacter*. There is no information on the survival of *Campylobacter* in composting systems. The most important agents for animals are those on the OIE List A and particularly Foot and Mouth Disease Virus and Classical Swine Fever Virus.
- There is generally a lack of information on the survival of viruses during composting.
- It has been suggested that compost may be responsible for an increase in cases of intoxication by the toxins of *Clostridium botulinum*.

Recommendations for further research work

- Determine the animal and human pathogens of concern to high-quality compost end-users by consulting with users of these products

- The temperature-time eradication conditions of the following human and animal pathogens should be determined in controlled composting experiments under UK conditions:
  E. coli
  Enterohaemorrhagic *E. coli* O157:H7
  Salmonella sp
  Clostridium perfringens
  Consideration should be given to determining the survival of OIE List A pathogens not normally endemic in the UK and particularly Foot and Mouth Disease Virus and Classical Swine Fever Virus.

- To facilitate experiments strains of *E. coli* and *Salmonella* with antibiotic resistance markers should be used. This will allow their isolation and differentiation from naturally-occurring strains.
  It will not be possible to use fully-virulent *E. coli* O157:H7 because this would require use of Category 3 (ACDP) facilities. Strains from which shiga toxin genes have been removed can be used at ACDP Category 2.

- Determine time-temperature profiles for the removal of human and animal pathogens in industrial-scale composting processes and best practices in terms of sanitation. Temperature profiles should be obtained from different windrow and in-vessel systems using green waste and vegetable waste feedstocks. This will enable the sanitary requirements determined in this review and the proposed research to be compared with what can be achieved on an industrial scale. Because the animal and human pathogens recommended for study are ACDP- and DEFRA-listed pathogens at Categories 2, 3 and 4 this recommendation may not be possible. It may be possible to validate processes using precisely-attenuated and vaccine strains of some of these agents.

- Disseminate the information obtained from this research to commercial compost producers and end-users.

- Determine the occurrence and survival of *C. botulinum* in composting systems to enable an assessment of possible disease risks. These have probably been over-emphasised.

Table 1: Possible occurrence of micro-organisms pathogenic for man and animals in biowaste of households

Bacteria	Viruses	Parasites
Salmonella	Enteroviruses	*Taenia*
E. coli	Hepatitis A	*Ascaris*
Enterobacter	Poliomyelitis*	
Yersinia	Coxsackieviruses	
Streptococcus	Echoviruses	
Proteus	Reoviruses	
Pseudomonas	Adenoviruses	
Klebsiella	Parvoviruses	
Citrobacter	Pestiviruses	

* This virus is due for eradication in 2004

From; Assman, E (1992); De Bertoldi *et al.*, 1998; Mayr, A, (1979); Moene, JR and Rheinthaler (1985)

Table 2: Examples of diseases which may be transmitted by animal wastes

Bacterial	**Rickettsial**
Anthrax	Q Fever
Brucellosis*	
Campylobacteriosis	**Fungal**
Colibacillosis	Coccidiomycosis Histoplasmosis
Erysipelas	
Johne's Disease	**Parasitic**
Tuberculosis	Ascariasis Trichuriasis
Leptospirosis	Fascioliasis
Salmonellosis	**Viral**
Swine dysentery	Foot and Mouth Disease* Newcastle Disease*
Tetanus	Swine Vesicular Disease* Transmissible gastroenteritis Viral enteritis

* Not endemic in UK – may be encountered.

(Jones, 1982)

Table 3. Some bacteria found in biosolids and diseases they transmit

Bacterium	Disease
Salmonella (approximately 1,700 types)*	salmonellosis gastroenteritis
Salmonella typhi +	typhoid fever
Mycobacterium tuberculosis	tuberculosis
Shigella sp.	shigellosis bacterial dysentery gastroenteritis
Campylobacter jejuni **	gastroenteritis
E. coli (pathogenic strains)	gastroenteritis
Yersinia sp.	yersiniosis
Vibrio cholerae +	cholera

+ Not endemic in UK

* There are actually more than 2,400 serotypes

** other species such as *C. coli* are also important

(Epstein, 2002)

Table 4. Some viruses found in biosolids and diseases they transmit.

Virus	Disease
Adenovirus (31 types)	Conjunctivitis Respiratory infections Gastroenteritis Poliomyelitis*
Coxsackievirus	Aseptic meningitis Gastroenteritis
Echovirus	Aseptic meningitis
Reovirus	Respiratory infections Gastroenteritis
Norwalk agents	Epidemic gastroenteritis
Hepatitis A virus	Infectious hepatitis
Rotavirus	Gastroenteritis Infant diarrhoea

* due for world eradication in 2004

(Epstein, 2002)

Table 5. Some protozoa and helminth parasites found in biosolids and the diseases they transmit

Protozoa	
Entamoeba histolytica	amoebic dysentery, amoebiasis acute enteritis
Giardia lamblia	giardiasis, diarrhoea
Balantidium coli	balantidiasis, diarrhoea, dysentery
Cryptosporidium	gastroenteritis
Toxoplasma gondii	toxoplasmosis
Helminths – Nematodes	
Ascaris suum	fever, respiratory effects
Ascaris lumbricoides	ascariasis, digestive and nutritional disturbances, abdominal pain, vomiting
Ancylostoma duodenale	hook worm disease, ancylostomatitis
Necator americanus	hook worm disease
Enterobius vermicularis	enterobiasis, intestinal inflammation, mucosal necrosis
Strongyloides stercoalis (threadworm)	strongyloidiasis, abdominal pain, diarrhoea
Toxocara canis (dog roundworm)	fever, abdominal pain, neurological symptoms
Trichuris trichuria (whip worm)	trichuriasis, abdominal pain, diarrhoea, anaemia
Helminths – Cestodes	
Taenia saginata (beef tapeworm)	taeniasis
Taenia solium (pork tapeworm)	taeniasis
Hymenolepsis (dwarf tapeworm)	taeniasis

(Epstein, 2000)

Note: many of these diseases are not endemic in the UK and are only encountered in UK citizens following travel.

Table 6. Number of pathogenic organisms in Food Waste, Yard Waste and Wood Waste

Organism	Food waste	Yard waste	Yard waste and Paper waste	Wood waste
		Indicator Organism:		
Total coliform	$5.0x10^6$	$8.0x10^5$	$5.0x10^5$	$1.3x10^6$
Faecal coliform	$2.0x10^4$	$8.0x10^5$	$5.0x10^5$	$1.3x10^6$
E. coli	$3.5x10^3$	$8.0x10^5$	$3.0x10^5$	$1.3x10^6$
Faecal *Streptococcus*	$8.0x10^6$	$1.6x10^6$	$1.6x10^6$	$1.6x10^6$
Enterococcus	$1.3x10^5$	$2.3x10^5$	$1.3x10^5$	$3.0x10^5$
		Pathogens:		
Salmonella sp.	<0.002	<0.002	0.36	<0.002
Staphylococcus	32.2	0.8	4.4	3.8
Listeria sp.	<0.02	<0.02	<0.02	<0.02
Parasites	protozoa	negative	negative	negative

(Epstein 2002; from E and A Consultants Inc., 1994)

Table 7. Some pathogens in municipal waste solid and diseases they can cause

Organism	Disease
Hepatitis virus	hepatitis
Staphylococcus sp.	skin infections osteomyelitis pneumonias impetigo
Diplococcus pneumoniae	pneumonia osteomyelitis arthritis endocarditis
Arenavirus	haemorrhagic fever
Neisseria meningitidis	meningococcal meningitis
Arboviruses	HIV, hepatitis encephalitis
Clostridium tetani	tetanus
Mycobacterium tuberculosis	pulmonary tuberculosis

(Epstein 2002; adapted from Geyer, 1994)

Note: the transmission of many of these diseases *via* wastes is very tenuous.

Table 8. Thermal Death Rates of some common pathogens and parasites.

Organism	**50°C**	**55°C**	**60°C**
Salmonella typhosa	-	30min	20min
Salmonella sp	-	60min	15-20min
Shigella sp	-	60min	-
Escherichia coli	-	60min	15-20min
Streptococcus pyogenes	-	10min	-
Mycobacterium diphtheriae	-	45min	-
Brucella abortus or *suis*	-	60min	3min
Entamoeba histolytica (cysts)	-	1sec	-
Trichinella spiralis	-	-	1sec
Necator americanus	50min	-	-
Ascaris lumbricoides	-	60min	-

(Day and Shaw, 2000)

Note: the names of organisms in this table do not follow normal nomenclature.

Table 9. Temperature-time relationship required for the destruction of several pathogens

Organism	Time (in minutes) for the destruction of organisms at several temperatures				
	50°C	**55°C**	**60°C**	**65°C**	**70°C**
Bacteria					
Salmonella typhi	-	-	30	-	4
E. coli	-	-	60	-	5
Mycobacterium tuberculosis	-	-	-	-	20
Shigella sp.	60	-	-	-	-
Mycobacterium diphtheriae	-	45	-	-	4
Brucella abortus *	-	60	-	3	-
Corynebacterium diphtheriae	-	45	-	-	4
Viruses					
Viruses	-	-	-	-	25
Protozoa					
Entamoeba histolytica cysts	5	-	-	-	-
Helminths					
Ascaris lumbricoides eggs	60	7	-	-	-
Necator americanus *	50	-	-	-	-
Taenia saginata	-	-	-	-	5

* Does not occur in UK

(Stern, 1974)

Table 10. Time-temperature as indicated by D-values for destruction of various microorganisms by heat.

Organism	D-values (min at given temperatures)	
	55°C	60°C
Adenovirus 12 NAIAD	11	0.17
Poliovirus, type 1	32	19
Ascaris ova	-	1.3
Histolytica cysts	44	25
Salmonella senftenberg 775W	89	7.5
Bacteriophage t2	267	47

(From Burge, 1983)

Note: The D-value (min) is the amount of time required to cause a 10-fold (one log) reduction in the number of organisms.

Table 11: Thermal death points of certain disease-carrying organisms in Man

Organism	Time / temperature °C	
Salmonella typhi	30min	55-60
Salmonella sp.	15-20min	60
	1 hour	55
Escherichia coli	15-20min	60
	1 hour	55
Brucella abortus	3min	61
Staphylococcus aureus	10min	50
Streptococcus pyogenes	10min	54
Mycobacterium tuberculosis	15-20min	66
Mycobacterium diphtheriae	45min	55
Shigella sp.	1 hour	55
Entamoeba histolytica	not stated	68
Taenia saginata	5min	71
Trichinella spiralis	1 hour	62-72
Necator americanus	50min	45

From Golueke (1982).

Table 12: Temperature/time requirements for the hygienic control of biowaste composting plants in various countries

Country		Temperature/Time relations	
Austria		>60-65°C	6 days
Denmark		>55°C	14 days
Germany	Open windrow	>55°C	14 days
		or 65°C	7 days
	Enclosed windrow	>60°C	7 days
Italy		65°C	24-36 days consecutive
Switzerland		>55°C	3 weeks
		>60°C	1 week
United States			
Sewage sludge biosolids			
Process to significantly reduce solids:			
		>40°C	>5 days windrow composting
		>55°C	4 hours during these 5 days
Process to further reduce pathogens:			
Static pile or in-vessel		>55°C	>3 days
Windrow		>55°C	>15 days with at least 5 turnings

(from Strauch, 1996)

Table 13: Preliminary list of possible indicator organisms for human and animal pathogens

Bacteria:	*Bacillus* sp
	Campylobacter sp
	Clostridium perfringens
	Escherichia coli
	Enterococcus
	Listeria sp
	Mycobacterium tuberculosis
	Mycobacterium paratuberculosis
	Salmonella sp
	Salmonella senftenberg W775
	Yersinia enterocolitica
Viruses:	Coliphages
	Coxackie B
	Parvovirus
Parasites:	Infective parasite eggs
	Ascaris sp
	Taenia sp
	Giardia lamblia
	Cryptosporidium parva

(from Anon, 2000; Sanitary Aspects of Composting Biodegradable waste)

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